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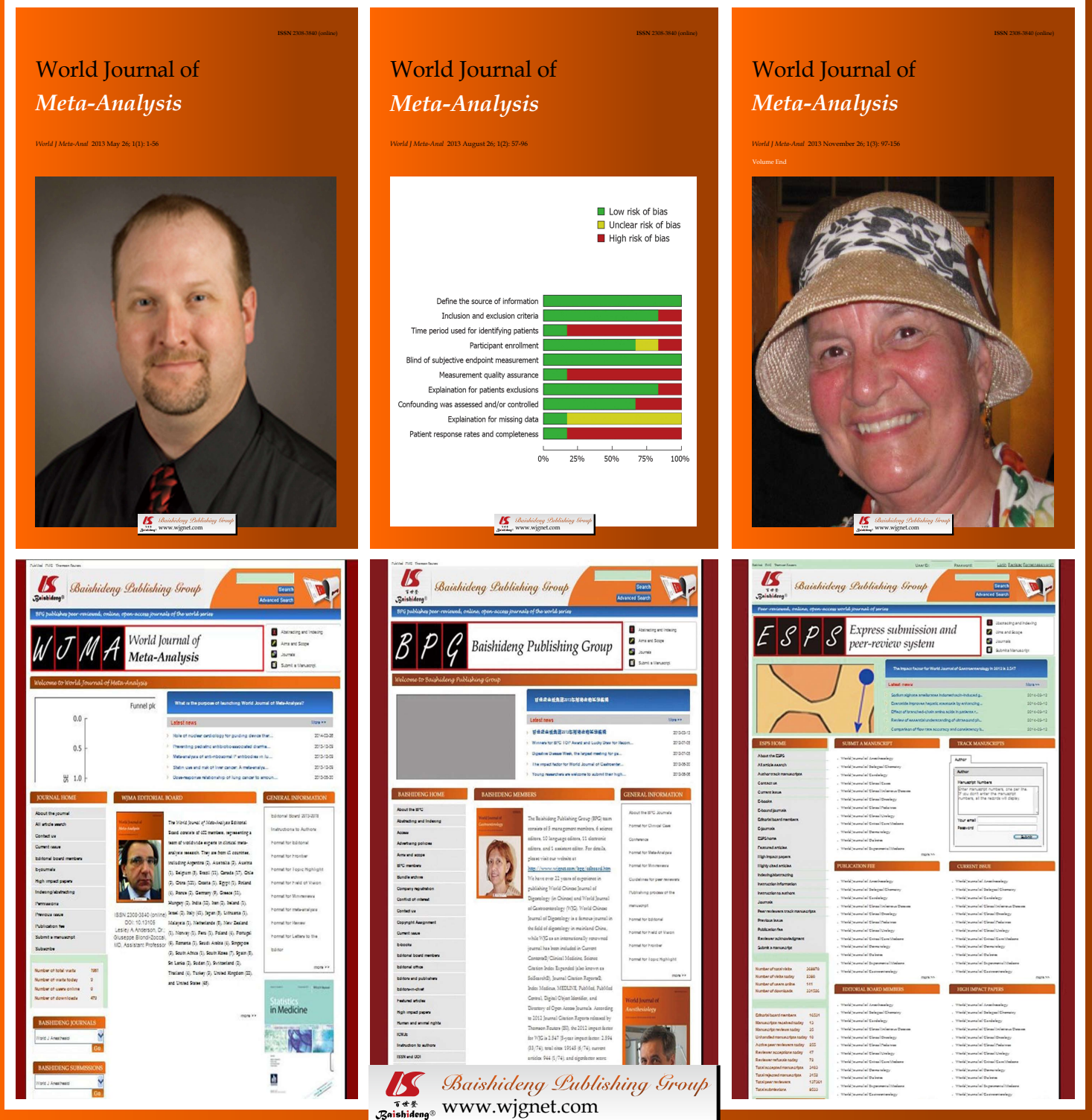

WJMA World Journal of Meta-Analysis

A peer-reviewed, online, open-access journal of meta-analysis

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2013-2018

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EDITORIAL

What is the purpose of launching *World Journal of Meta-Analysis*?

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Abstract

The exponential growth of scientific evidence (*i.e.*, primary research) and the ongoing development of methods to summarize such evidence, such as meta-analyses and mixed treatment comparisons (*i.e.*, secondary research), make the worldwide dissemination of high-quality meta-analyses and pertinent articles a key scientific priority. The *World Journal of Meta-Analysis* will apply an electronic open access publishing approach combined with a timely and thorough peer-review of submitted manuscripts, weighing more on quality than priority, in order to improve the dissemination of systematic reviews and meta-analyses, as well as novelties and advancements in methods related to them, focusing on clinical medicine, but spanning all biomedical, epidemiological, and psychological research fields.

Key words: Evidence-based medicine; Meta-analysis; Meta-regression; Review; Systematic review

Core tip: The exponential growth of scientific evidence and the ongoing development of meta-analyses make the worldwide dissemination of the latter study type a key scientific priority. The *World Journal of Meta-Analysis* will apply an electronic open access publishing approach, in order to improve the dissemination of systematic reviews and meta-analyses, focusing on clinical medicine, but spanning all biomedical, epidemiological, and psychological research fields.

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INTRODUCTION

The scientific literature includes thousands of journals on a extremely wide variety of topics, stemming from scientific methods (*e.g.*, *Bayesian Analysis*) or techniques (*e.g.*, *Magnetic Resonance Imaging*) to specific clinical topics (*e.g.*, *Stroke*). In an era dominated by online bibliometric resources and fast dissemination and accrual of scientific evidence, it is becoming increasingly difficult to remain abreast of the most recent scholarly developments. This is one of the main reasons for the success of secondary research, *i.e.*, any form of scholarly activity which aims to appraise and summarize specific research publications (*i.e.*, primary research)[1,2].

Within the context of secondary research, qualitative reviews, defined as viewpoints summarizing the evidence base on a specific scientific topic, conducted without any explicit or validated method, are commonplace. Conversely, systematic reviews are based on explicit and, when possible, validated means to search, select, appraise and summarize the evidence base on a specific scientific topic. Meta-analysis is the method by which primary data, given appropriate methodological approaches, can be summarized, and it is best undertaken in the context of a sys-

tematic retrieval of the literature[3]. Finally, more advanced types of secondary research endeavors include meta-regression analyses, cumulative meta-analyses, individual patient-level meta-analyses, overview of systematic reviews, and mixed treatment comparisons. The latter study type, also known as network meta-analyses, appears very promising and, despite obvious methodological limitations which still require ample research, capable of powerful evidence synthesis[4-8].

The success of reviews, systematic reviews, and meta-analyses is well testified by the fact that this research design has grown exponentially in recent decades, outpacing, at least in relative terms, all other research designs, and it is the most likely to be quoted once published[9-11]. Despite such ongoing success and impact among both researchers and readers, until recently journals devoted specifically to publishing systematic reviews and meta-analyses were lacking. However, with the creation of *Systematic Reviews* in February 2012[12], and the birth of the *World Journal of Meta-Analysis* (*World J Meta-Anal*, *WJMA*, ISSN 2308-3840, DOI: 10.13105) today, accessibility and retrieval of important, and peer-reviewed meta-analyses are set to improve. The *WJMA* Editorial Board has now been established and consists of 402 distinguished experts from 41 countries.

It is not casual that both journals are seeing their light within the electronic open access publication framework. This novel approach, unheard of just a decade ago, is revolutionizing the way evidence is created and disseminated, by putting increasing emphasis on readers downloading, using and commenting on articles, in addition to other researchers later studying and quoting them, rather than on peer-reviewers and editors, who are used to appraise them before full publication. This paradigm shift is well exemplified by the ongoing success of *PLOS ONE*, an open access journal published without any editorial regard for priority. In such scenario, we strongly believe that meta-analyses and similar scholarly efforts to summarize scientific evidence will become more and more important, and thus merit a specific and protected scholarly haven. This is what we, as Editors-in-Chief of the *WJMA*, strive to do.

Among the key advantages of meta-analyses are the cost-effectiveness, ability to maximize statistical power, bolster external validity, appraise clinical and statistical consistency, and explore effect modifiers or moderators, including small study effects (*e.g.*, publication bias) and important patient or study features[13,14]. Despite such important pros, meta-analyses have been criticized as well, citing among the potential disadvantages the inability to correct flaws already present in the original studies, the risks of ecological fallacy and spurious precision, and the fact that an average effect estimate may not be easily applicable to the individual case which is faced in real-world practice[15]. Despite these important drawbacks, it is clear that researchers and readers worldwide trust meta-analyses as a reasonably sound and rigorous research design, and the ongoing accumulation of new methods and refinements in the underlying statistical methods will improve them further, bolstering our optimism concerning their current and future scholarly role.

AIM AND SCOPE

WJMA is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians, with a specific focus on meta-analysis, systematic review, mixed-treatment comparison, meta-regression, and overview of reviews.

The primary task of *WJMA* is to rapidly publish high-quality basic research, clinical studies, methodology or scientific theory in diverse areas of biomedical sciences, Editorial, Frontier, Field of Vision, Minireviews, Review, Topic Highlight, Medical Ethics, and Meta-Analysis. *WJMA* covers a variety of clinical medical fields including allergy, anesthesiology, cardiac medicine, clinical genetics, clinical neurology, critical care, dentistry, dermatology, emergency medicine, endocrinology, family medicine, gastroenterology and hepatology, geriatrics and gerontology, hematology, immunology, infectious diseases, internal medicine, obstetrics and gynecology, oncology, ophthalmology, orthopedics, otolaryngology, pathology, pediatrics, peripheral vascular disease, psychiatry, radiology, rehabilitation, respiratory medicine, rheumatology, surgery, toxicology, transplantation, and urology and nephrology, while maintaining its unique dedication to systematic reviews and meta-analyses.

WJMA is dedicated to become an influential and prestigious journal in meta-analysis, to promote the development of the above disciplines, and to improve the diagnostic and therapeutic skills and expertise of clinicians.

WJMA is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 42 open access clinical medical journals, and is one of the leading medical publishers, with first-class editing and publishing capacity and production.

CONTENTS OF PEER REVIEW

In order to guarantee the quality of articles published in the journal, *WJMA* usually invites three experts to comment on the submitted papers. The contents of peer review include: (1) whether the contents of the manuscript are of great importance and novelty; (2) whether the study is complete and described clearly; (3) whether the discussion and conclusion are justified; (4) whether the citations of references are necessary and reasonable; and (5) whether the presentation and use of tables and figures are correct and complete.

COLUMNS

The columns in the issues of *WJMA* will include: (1) Editorial: The editorial board members are invited to make

comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than 2 years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented will be provided as well (including authors, article title, journal name, year, volume, and inclusive page numbers; (4) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research to provide readers; (5) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (6) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic; (7) Meta-Analysis: Covers the systematic review, mixedtreatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, *e.g.*, the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (8) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (9) Letters to the Editor: To discuss and make reply to the contributions published in *WJMA*, or to introduce and comment on a controversial issue of general interest; (10) Book Reviews: To introduce and comment on quality monographs; and (11) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

THE CASE FOR THE *WJMA*

So, who would benefit from submitting a manuscript to the *WJMA* and who should read it? Anyone reporting a meta-analysis, systematic review, mixed-treatment comparison, meta-regression, overview of reviews, or network meta-analysis in any medical-related field is invited to submit his or her work to the *WJMA*. This holds also true for anyone wishing to publish the protocol of any of the above studies, but also for all authors who want to discuss meta-analyses published elsewhere, or exploit meta-analytic methods to appraise other important scientific issues, such as is done in meta-epidemiologic enquiries. Manuscripts focusing on meta-analytic methods are also welcome as developments and improvements in the way meta-analyses are conducted and reported occur with increasing frequency. Indeed, our mission is also to make presentation of results of meta-analyses more easily understandable by the reader. This goal might be achieved by explicitly publishing technical papers, which could also be in the form of simple and clear education papers. While the Editors-in-Chief are skilled and practice routinely clinical medicine and epidemiology, the *WJMA* aims for a broader scope, which build upon its key interest in clinical medicine to include also all biomedical, epidemiological, and psychological research fields.

Accordingly, anyone interested in meta-analyses or important novelties or advancements related to them within the context of clinical medicine, as well as biomedical, epidemiological, and psychological topics, should read regularly the *WJMA*. Moreover, this journal will prove useful also for anyone wanting a high-quality synthesis of information, such that they do not need to trawl the literature themselves as it will already be summarized for them. As Editors-in-Chief, we will surely enjoy our involvement in this exciting editorial effort, and make a formal oath that thorough yet timely external peer-review will be the rule to all manuscripts received, and that quality will always have the upper hand on priority in shaping the editorial decision.

CONCLUSION

In conclusion, the *WJMA* aims to provide for both authors and readers a friendly yet authoritative scholarly framework for the dissemination of meta-analyses and important scientific advancements related to them within the field of medicine, as well as all ancillary disciplines, in keeping with the comprehensive effort of improving dissemination of high-quality science by BPG.

REFERENCES

1 **Biondi-Zoccai G**, Landoni G, Modena MG. A journey into

clinical evidence: from case reports to mixed treatment comparisons. *HSR Proc Intensive Care Cardiovasc Anesth* 2011; **3**: 93-96 [PMID: 23441269]
2 **Biondi-Zoccai G**, Lotrionte M, Landoni G, Modena MG. The rough guide to systematic reviews and meta-analyses. *HSR Proc Intensive Care Cardiovasc Anesth* 2011; **3**: 161-173 [PMID: 23439862]
3 **Biondi-Zoccai GG**, Abbate A, Sheiban I. Systematic reviews and meta-analyses "For Dummies". *EuroIntervention* 2009; **5**: 289-291 [PMID: 19736151 DOI: 10.4244/A46]
4 **Biondi-Zoccai GG**, Agostoni P, Abbate A, Testa L, Burzotta F, Lotrionte M, Crea F, Biasucci LM, Vetrovec GW, Colombo A. Adjusted indirect comparison of intracoronary drug-eluting stents: evidence from a metaanalysis of randomized bare-metal-stent-controlled trials. *Int J Cardiol* 2005; **100**: 119-123 [PMID: 15820294 DOI: 10.1016/j.ijcard.2004.11.001]
5 **Biondi-Zoccai G**, Lotrionte M, Agostoni P, Abbate A, Romagnoli E, Sangiorgi G, Angiolillo DJ, Valgimigli M, Testa L, Gaita F, Sheiban I. Adjusted indirect comparison meta-analysis of prasugrel versus ticagrelor for patients with acute coronary syndromes. *Int J Cardiol* 2011; **150**: 325-331 [PMID: 20828843 DOI: 10.1016/j.ijcard.2010.08.035]
6 **Palmerini T**, Biondi-Zoccai G, Della Riva D, Stettler C, Sangiorgi D, D'Ascenzo F, Kimura T, Briguori C, Sabatè M, Kim HS, De Waha A, Kedhi E, Smits PC, Kaiser C, Sardella G, Marullo A, Kirtane AJ, Leon MB, Stone GW. Stent thrombosis with drug-eluting and bare-metal stents: evidence from a comprehensive network meta-analysis. *Lancet* 2012; **379**: 1393-1402 [PMID: 22445239 DOI: 10.1016/S0140-6736(12)60324-9]
7 **Chatterjee S**, Biondi-Zoccai G, Abbate A, D'Ascenzo F, Castagno D, Van Tassell B, Mukherjee D, Lichstein E. Benefits of β blockers in patients with heart failure and reduced ejection fraction: network meta-analysis. *BMJ* 2013; **346**: f55 [PMID: 23325883 DOI: 10.1136/bmj.f55]
8 **Biondi-Zoccai G**, Frati G, D'Ascenzo F, Stone GW, Lotrionte M, Palmerini T. Network meta-analyses and mixed treatment comparisons: Are they true scientific endeavors? *Int J Cardiol* 2013; Epub ahead of print [PMID: 23410483 DOI: 10.1016/j.ijcard.2013.01.054]
9 **Biondi-Zoccai GG**, Lotrionte M, Abbate A, Testa L, Remigi E, Burzotta F, Valgimigli M, Romagnoli E, Crea F, Agostoni P. Compliance with QUOROM and quality of reporting of overlapping meta-analyses on the role of acetylcysteine in the prevention of contrast associated nephropathy: case study. *BMJ* 2006; **332**: 202-209 [PMID: 16415336 DOI: 10.1136/bmj.38693.516782.7C]
10 **Patsopoulos NA**, Analatos AA, Ioannidis JP. Relative citation impact of various study designs in the health sciences. *JAMA* 2005; **293**: 2362-2366 [PMID: 15900006 DOI: 10.1001/jama.293.19.2362]
11 **Zambon M**, Biondi-Zoccai G, Bignami E, Ruggeri L, Zangrillo A, Landoni G. A comprehensive appraisal of meta-analyses focusing on nonsurgical treatments aimed at decreasing perioperative mortality or major cardiac complications. *J Anesth* 2012; **26**: 509-515 [PMID: 22476532 DOI: 10.1007/s00540-012-1372-z]
12 **Moher D**, Stewart L, Shekelle P. Establishing a new journal for systematic review products. *Syst Rev* 2012; **1**: 1 [PMID: 22587946 DOI: 10.1186/2046-4053-1-1]
13 **Thompson SG**, Pocock SJ. Can meta-analyses be trusted? *Lancet* 1991; **338**: 1127-1130 [PMID: 1682553]
14 **Pogue J**, Yusuf S. Overcoming the limitations of current meta-analysis of randomised controlled trials. *Lancet* 1998; **351**: 47-52 [PMID: 9433436 DOI: 10.1016/S0140-6736(97)08461-4]
15 **Lau J**, Ioannidis JP, Schmid CH. Summing up evidence: one answer is not always enough. *Lancet* 1998; **351**: 123-127 [PMID: 9439507 DOI: 10.1016/S0140-6736(97)08468-7]

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EDITORIAL

Meta-analyses in the wonderland of neurology

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Abstract

Meta-analyses are often misused and underused in neurology. This editorial provides some comments on the role of meta-analyses in neurological research. Recently, a huge increase in the number of meta-analyses and systematic reviews has been observed in neurological journals. The major strengths of meta-analyses are the increase of statistical power. However, as for any other investigative tool, meta-analytic research is a research method itself which can produce severe shortcomings. Specifically, the issues of search terms, time periods of published studies, databases used for searching, the definitions of inclusion and exclusion criteria for papers (which greatly affect clinical heterogeneity), publication bias; and the statistical methods used, dramatically influence the results of meta-analyses. The main problem of meta-analyses is that they cannot be expected to overcome the limitations of the studies they include (the so-called "garbage in, garbage out" phenomenon). Furthermore, most systematic reviews in the neurological literature lead to the unsatisfying and clinically frustrating statement "further studies are needed". However it is much more frustrating to see how the gaps in scientific knowledge identified by meta-analyses have not been translated into serious efforts to fill them. Besides their role in evaluating efficacy and tolerability of drugs, meta-analyses may be used to assess diagnostic values of debatable clinical findings, as they represent powerful tools to try to answer questions not posed by individual studies and to settle controversies arising from conflicting claims.

Key words: Clinical evaluation; Epilepsy; Meta-analysis; Migraine; Neurology

Core tip: Besides their role in evaluating efficacy and tolerability of drugs, meta-analyses may be used to assess diagnostic values of debatable clinical findings, as they represent powerful tools to try to answer questions not posed by individual studies and to settle controversies arising from conflicting claims.

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META-ANALYSES AND THE NEUROLOGICAL UNANSWERED QUESTIONS

It is both astonishing and frustrating to consider how much meta-analyses are misused and underused in neurological research.

As a young neurology resident, I used to consider experienced neurologists as enlightened, trustworthy and

truth holding people. After gaining some clinical experience in neurology and in evidence-based practice, I learnt to mistrust self assured people without worries, as I realized that truth does not exist in medicine, as it is an asymptotic process[1], not a divine revelation. Similarly, certainty in medicine does not exist, only probability does.

Neurology is probably the field of medicine most burdened with dilemmas on several crucial aspects of pathophysiology, diagnosis, and treatment of a number of diseases. Consider for instance the pathophysiological mechanisms involved in Alzheimer's disease, the questionable therapeutical strategies against multiple sclerosis, and the endless discussions on cortical excitability in migraine or epilepsy. Questions in neurology seem to be much more than answers and as for the Holy Grail, the quest for definite conclusions is hard to be achieved but nevertheless remains an urgent need.

NEUROLOGICAL META-ANALYSES: REASONS FOR SUCCESS

Recently, a huge increase in the number of meta-analyses and systematic reviews has been observed in neurological journals. For instance, the number of articles published in four major neurological journals (*Brain*; *Annals of Neurology*; *Neurology*; and *Journal of Neurology, Neurosurgery, and Psychiatry*) increased from only 53 (1993-2002) to 187 (2003-2012)!

Such a great proliferation of meta-analyses may be easily understood: because of its inflated sample size, meta-analyses can detect treatment effects with greater statistical power, estimating these effects with greater precision than any single study.

SOME PITFALLS OF META-ANALYSES

However, improper use of meta-analyses may lead to erroneous conclusions regarding treatment efficacy. In fact, as for any other investigative tool, meta-analysis is a research method itself which can produce severe shortcomings.

Specifically, the issues of search terms, time periods of published studies, databases used for searching, the definitions of inclusion and exclusion criteria of papers (which greatly affect clinical heterogeneity), publication bias; and the statistical methods used, dramatically influence the results of meta-analyses.

Readers should be well aware of these pitfalls. After all, meta-analyses are human constructs, and as such they are fallible. All that glitters isn't gold!

The main problem of meta-analyses is that they cannot be expected to overcome the limitations of the studies they include (the so-called "garbage in, garbage out" phenomenon). Furthermore, most systematic reviews in neurological literature lead to the unsatisfying and clinically frustrating statement "further studies are needed". However, it is much more frustrating to see how the gaps in scientific knowledge identified by meta-analyses have not been translated into serious efforts to fill them.

ROLE OF META-ANALYSES IN NEUROLOGICAL RESEARCH: SOME PERSONAL EXAMPLES

Despite the above mentioned risks of pitfalls, how can meta-analyses help neurologists in their quest for answers?

As a neurologist dealing with epilepsy and clinical neurophysiology I learnt to use meta-analyses as powerful tools to try to answer questions not posed by individual studies and to settle controversies arising from conflicting claims.

Meta-analyses allowed me not only to evaluate efficacy and tolerability of some neurological treatments[2,3], but also to better understand the diagnostic utility of some debatable clinical findings such as tongue biting, urinary incontinence or eye closure in the differential diagnosis of seizures[4-6]. Meta-analyses helped me to shed further light on the role of cortical excitability in the pathophysiology of migraine or idiopathic generalized epilepsies[7-10].

Finally, meta-analysis prompted me to consider the one single point of view of the view of one single point.

Dear neurologists, if there is no answer, just look for it! And may meta-analyses give you a hand!

REFERENCES

1 **Brigo F**. An evidence-based approach to proper diagnostic use of the electroencephalogram for suspected seizures. *Epilepsy Behav* 2011; **21**: 219-222 [PMID: 21624850 DOI: 10.1016/j.yebeh.2011.04.004]

2 **Brigo F**, Del Felice A. Melatonin as add-on treatment for epilepsy. *Cochrane Database Syst Rev* 2012; **6**: CD006967 [PMID: 22696363 DOI: 10.1002/14651858.CD006967.pub2]

3 **Brigo F**, Storti M, Del Felice A, Fiaschi A, Bongiovanni LG. IV Valproate in generalized convulsive status epilepticus: a systematic review. *Eur J Neurol* 2012; **19**: 1180-1191 [PMID: 22182304 DOI: 10.1111/j.1468-1331.2011.03606.x]

4 **Brigo F**, Nardone R, Bongiovanni LG. Value of tongue biting in the differential diagnosis between epileptic seizures and syncope. *Seizure* 2012; **21**: 568-572 [PMID: 22770819 DOI: 10.1016/j.seizure.2012.06.005]

5 **Brigo F**, Storti M, Lochner P, Tezzon F, Fiaschi A, Bongiovanni LG, Nardone R. Tongue biting in epileptic seizures and psychogenic events: an evidence-based perspective. *Epilepsy Behav* 2012; **25**: 251-255 [PMID: 23041172 DOI: 10.1016/j.yebeh.2012.06.020]

6 **Brigo F**, Nardone R, Ausserer H, Storti M, Tezzon F, Manganotti P, Bongiovanni LG. The diagnostic value of urinary incontinence in the differential diagnosis of seizures. *Seizure* 2013; **22**: 85-90 [PMID: 23142708 DOI: 10.1016/j.seizure.2012.10.011]

7 **Brigo F**, Ausserer H, Nardone R, Tezzon F, Manganotti P, Bongiovanni LG. Clinical utility of ictal eyes closure in the differential diagnosis between epileptic seizures and psychogenic events. *Epilepsy Res* 2013; **104**: 1-10 [PMID: 23332582 DOI: 10.1016/j.eplepsyres.2012.12.004]

8 **Brigo F**, Storti M, Nardone R, Fiaschi A, Bongiovanni LG, Tezzon F, Manganotti P. Transcranial magnetic stimulation of visual cortex in migraine patients: a systematic review with meta-analysis. *J Headache Pain* 2012; **13**: 339-349 [PMID: 22535147 DOI: 10.1007/s10194-012-0445-6]

9 **Brigo F**, Storti M, Tezzon F, Manganotti P, Nardone R. Primary visual cortex excitability in migraine: a systematic review with meta-analysis. *Neurol Sci* 2012; Epub ahead of print [PMID: 23263736]

10 **Brigo F**, Storti M, Benedetti MD, Rossini F, Nardone R, Tezzon F, Fiaschi A, Bongiovanni LG, Manganotti P. Resting motor threshold in idiopathic generalized epilepsies: a systematic review with meta-analysis. *Epilepsy Res* 2012; **101**: 3-13 [PMID: 22542570 DOI: 10.1016/j.eplepsyres.2012.03.020]

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MINIREVIEW

Sirturo (Bedaquiline): The first new anti tubercular drug in decades

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Abstract

Tuberculosis (TB) is an infection caused by *Mycobacterium tuberculosis* and is one of the world's deadliest diseases. Multidrug resistant TB (MDR-TB) is a serious form of TB and it implies resistance for at least two essential first-line agents like, Isoniazid and Rifampicin. The US Food and Drug Administration (FDA) granted accelerated approval to Janseen Therapeutics "Sirturo (Bedaquiline)", a diarylquinoline anti mycobacterial drug on December 28, 2012 as part of combination therapy in adults (≥ 18 years) to treat MDR-TB when other alternatives are not available. The FDA also granted Sirturo fast track designation, priority review and orphan-product designation. Bedaquiline inhibits mycobacterial ATP (adenosine 5'-triphosphate) synthase, an enzyme that is essential for the generation of energy in *Mycobacterium tuberculosis*.

Key words: Multidrug resistant tuberculosis; Bedaquiline; Sirturo

Core tip: The US Food and Drug Administration granted accelerated approval to Janseen Therapeutics "Sirturo (Bedaquiline)", a diarylquinoline anti mycobacterial drug on December 28, 2012 as part of combination therapy in adults (≥ 18 years) to treat multidrug resistant tuberculosis when other alternatives are not available.

Undela K. Sirturo (Bedaquiline): The first new anti tubercular drug in decades. *World J Meta-Anal* 2013; 1(1): 8-9 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i1/8.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i1.8

INTRODUCTION

Tuberculosis (TB) is an infection caused by *Mycobacterium tuberculosis* and is one of the world's deadliest diseases. According to the Centers for Disease Control and Prevention, nearly 9 million people around the world and 10528 people in the United States became sick with TB in 2011.

Multidrug resistant TB (MDR-TB) is a serious form of TB and it implies resistance for at least two essential first-line agents, like Isoniazid and Rifampicin. MDR-TB is a possibly fatal disease that affects as many as 630000 people worldwide who cannot be cured with existing therapies alone and it is considered an orphan disease in the US, with 98 reported patients in 2011. The World Health Organisation estimates more than two million people will develop MDR-TB between 2011 and 2015.

The US Food and Drug Administration (FDA) granted accelerated approval to Janseen Therapeutics "Sirturo (Bedaquiline)"[1], a diarylquinoline anti mycobacterial drug on December 28, 2012 as part of combination therapy in adults (≥ 18 years) to treat MDR-TB when other alternatives are not available and it leads to the approval of the first TB therapy in 40 years with a new mechanism of action. The FDA also granted Sirturo fast track designation, priority review and orphan-product designation (Figure 1)[2].

MECHANISM OF ACTION

Bedaquiline inhibits mycobacterial ATP (adenosine 5'-tri-

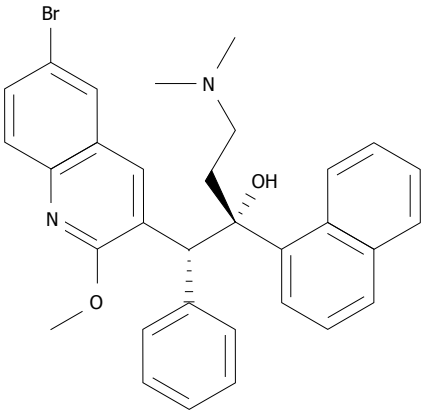

Figure 1 Chemical constitution of "Sirturo (Bedaquiline)".

phosphate) synthase, an enzyme that is essential for the generation of energy in *Mycobacterium tuberculosis*.

MECHANISMS OF RESISTANCE

Mycobacterial resistance mechanisms that affect Bedaquiline include modification of the atpE target gene. Not all isolates with increased minimum inhibitory concentrations have atpE mutations, suggesting the existence of at least one other mechanism of resistance.

SPECTRUM OF ACTIVITY

Bedaquiline has been shown to be active against most isolates of *Mycobacterium tuberculosis*.

SAFETY AND EFFECTIVENESS

Bedaquiline's safety and effectiveness were established in 440 patients in two phase 2 clinical trials. Patients in the first trial were randomly assigned to be treated with Sirturo plus other drugs used to treat MDR-TB (Sirturo treatment group) (n = 79), or a placebo plus other drugs used to treat MDR-TB (placebo treatment group) (n = 81); the other drugs used to treat MDR-TB consisted of a combination of five other antimycobacterial drugs (ethionamide, kanamycin, pyrazinamide, ofloxacin and cycloserine/terizidone or available alternative). Sirturo was administered as 400 mg once daily for the first 2 wk and as 200 mg three times per week for the following 22 wk. After the 24 wk study drug (Sirturo or placebo) treatment phase, patients continued to receive their other drugs used to treat MDR-TB until total treatment duration of 18 to 24 mo was achieved, or at least 12 mo after the first confirmed negative culture. All patients in the second trial, which is ongoing, received Sirturo plus other MDR-TB drugs. In both studies, the primary endpoint was time to sputum culture conversion (SCC), defined as the interval in days between the first dose of the study drug and the date of the first of two consecutive negative sputum cultures collected at least 25 d apart during treatment.

Results from the first trial showed that patients treated with Sirturo combination therapy achieved SCC in a median time of 83 d, compared with 125 d in patients treated with placebo combination therapy. According to these results, 77.6% of patients in the treatment group reached treatment success after 24 wk compared with 57.6% of those in the placebo group. Results from the second trial showed the median time to SCC was 57 d, supporting the efficacy findings of the first trial.

ADVERSE DRUG REACTIONS

Sirturo carries a boxed warning, alerting patients and health care professionals that the drug can affect the heart's electrical activity (QT prolongation) and also notes that an increased risk of death was seen in the Sirturo treatment group (9/79, 11.4%) compared to the placebo treatment group (2/81, 2.5%) in one placebo-controlled trial. Sirturo should only be used when an effective treatment regimen cannot otherwise be provided.

The most common adverse reactions reported in > 10% of patients treated with Sirturo are nausea, arthralgia and headache. Additional adverse events reported in ⩾ 10% of patients treated with Sirturo and with a higher frequency than the placebo treatment group are hemoptysis and chest pain. More hepatic-related adverse drug reactions were reported with the use of Sirturo plus other drugs used to treat TB compared to other drugs used to treat TB without the addition of Sirturo.

The safety and efficacy of Sirturo for the treatment of drug-sensitive TB has not been established. In addition, there is no data on the treatment with Sirturo of extrapulmonary TB (*e.g.*, central nervous system).

Sirturo was discovered by researchers at "Janssen" and is currently under review by three regulatory bodies, including the European Medicines Agency (European Union), State Food and Drug Administration (China) and Medicines Control Council (South Africa).

REFERENCES

1 **Diacon AH**, Donald PR, Pym A, Grobusch M, Patientia RF, Mahanyele R, Bantubani N, Narasimooloo R, De Marez T, van Heeswijk R, Lounis N, Meyvisch P, Andries K, McNeeley DF. Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. *Antimicrob Agents Chemother* 2012; **56**: 3271-3276 [PMID: 22391540 DOI: 10.1128/AAC.06126-11]

2 US Food Drug Administration. Available from: URL: http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/204384s000lbl.pdf

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Ascorbic acid and low-volume polyethylene glycol for bowel preparation prior to colonoscopy: A meta-analysis

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Abstract

AIM: To evaluate the benefits of low-volume polyethylene glycol (PEG) with ascorbic acid compared to full-dose PEG for colonoscopy preparation.

METHODS: MEDLINE, Cochrane Central Register of Controlled Trials and Database of Systematic Reviews, CINAHL, PubMed, and recent abstracts from major conferences were searched (January 2012). Only randomized-controlled trials on adult subjects comparing low-volume PEG (2 L) with ascorbic acid *vs* full-dose PEG (3 or 4 L) were included. Meta-analysis for the efficacy of low-volume PEG with ascorbic acid and full-dose PEG were analyzed by calculating pooled estimates of number of satisfactory bowel preparations as well as adverse patient events (abdominal pain, nausea, vomiting). Separate analyses were performed for each main outcome by using OR with fixed and random effects models. Heterogeneity was assessed by calculating the I^2 measure of inconsistency. RevMan 5.1 was utilized for statistical analysis.

RESULTS: The initial search identified 242 articles and trials. Nine studies (n = 2911) met the inclusion criteria and were analyzed for this meta-analysis with mean age range from 53.0 to 59.6 years. All studies were randomized controlled trials on adult patients comparing large-volume PEG solutions (3 or 4 L) with low-volume PEG solutions and ascorbic acid. No statistically significant difference was noted between low-volume PEG with ascorbic acid and full-dose PEG for number of satisfactory bowel preparations (OR 1.07, 95%CI: 0.86-1.33, P = 0.56). No statistically significant difference was noted between low-volume PEG with ascorbic acid and full-dose PEG for abdominal pain (OR 1.09, 95%CI: 0.81-1.48, P = 0.56), nausea (OR 0.70, 95%CI: 0.49-1.00, P = 0.05), or vomiting (OR 0.99, 95%CI: 0.78-1.26, P = 0.95). No publication bias was noted.

CONCLUSION: Low-volume PEG with the addition of ascorbic acid demonstrates no statistically significant difference to full-dose PEG for satisfactory bowel preparation and side-effects.

Key words: Polyethylene glycol; Ascorbic acid; Colonoscopy; Meta-analysis; Bowel preparation

Core tip: Optimal visualization of the colon during colonoscopy requires adequate bowel preparation that is effective and tolerable to the patient. Low-volume polyethylene glycol (PEG) preparation coupled with ascorbic acid has been utilized to enhance patient tolerability without affecting the quality of bowel preparation. This

meta-analysis shows that bowel preparation with low-volume PEG with ascorbic acid does not differ from full-dose PEG for quality of bowel preparation or patient tolerability.

Godfrey JD, Clark RE, Choudhary A, Ashraf I, Matteson ML, Puli SR, Bechtold ML. Ascorbic acid and low-volume polyethylene glycol for bowel preparation prior to colonoscopy: A meta-analysis. *World J Meta-Anal* 2013; 1(1): 10-15 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i1/10.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i1.10

INTRODUCTION

Colorectal cancer (CRC) is the third-leading cause of cancer and second-leading cause of cancer-related deaths in the United States[1]. In 2012, it is estimated that 143460 new cases of CRC will be diagnosed and 51690 deaths will occur secondary to this disease[1]. Given these estimations, it has become increasingly important to screen for and prevent CRC, ideally detecting the disease in an early stage. Colonoscopy has become a widely available screening test for both preventing and detecting CRC and has been recommended as the preferred CRC prevention test by the American College of Gastroenterology (ACG)[2]. Furthermore, colonoscopy is an important tool in the work-up and management of various other conditions including inflammatory bowel disease, lower-gastrointestinal bleeding, and diarrhea[3-6].

To provide optimal visualization of the colonic mucosa during exam, colonoscopy is dependent on an adequate bowel preparation[7,8]. In order to accomplish this, patients are asked to drink, at times, large volumes of colon preparation solutions[9-11]. This large amount of oral intake prior to a colonoscopy can lead to patient discomfort, nausea, vomiting, and poor patient compliance, which, in turn, leads to a poor colon preparation and increased potential for missed lesions and need for repeat colonoscopy[12-14].

Several bowel cleansing preparations have been developed and used over the years. One of the most common preparations is polyethylene glycol (PEG) which was introduced in 1980[15]. The use of PEG generally requires the ingestion of a large volume of solution (usually 4 L). Several studies have investigated the utility of a low-volume PEG solution (2-3 L) with the addition of adjunct therapy such as a laxative or additive[16-18]. More specifically, some studies have compared a standard PEG preparation to a low-volume PEG preparation coupled with ascorbic acid, acting as an osmotic laxative[19-27]. The low-volume of PEG solution used in these studies has been theorized to decrease patient side-effects and improve patient compliance, resulting in a higher quality of bowel preparation. Therefore, we conducted a meta-analysis to compare low-volume PEG solution with ascorbic acid to standard volume PEG solution for bowel preparation for colonoscopy.

MATERIALS AND METHODS

Study selection criteria

All randomized controlled trials (RCTs) on adult patients comparing large-volume PEG solutions (3 or 4 L) with low-volume PEG solutions and ascorbic acid were included in our analysis.

Data collection and extraction

A three-stage search method was utilized to maximize search results. First, a comprehensive search was performed in MEDLINE, Cochrane Central Register of Controlled Trials and Database of Systematic Reviews, CINAHL, PubMed in January 2012. Second, references of the retrieved articles and reviews were manually searched for any additional articles. Third, a manual search of abstracts submitted to the Digestive Disease Week and the ACG national meetings was performed from 2003-2011. All articles were searched irrespective of language, publication status (articles or abstracts), or results. The search terms used were PEG and ascorbic acid. Only randomized-controlled trials on adult subjects that compared low-volume PEG (2 L) with ascorbic acid *vs* full-dose PEG (3 or 4 L) were included. Standard forms were used to extract data by two independent reviewers. Each study was evaluated by a Jadad score[28] and criteria based on Jüni *et al*[29] to assess the quality of the study.

Statistical analysis

A meta-analysis was performed comparing the efficacy of low-volume PEG with ascorbic acid and full-dose PEG by calculating pooled estimates of number of satisfactory bowel preparations as well as adverse patient events including abdominal pain, nausea, and vomiting. Separate analyses were performed for each main outcome by using OR with fixed and random effects models which was considered significant if $P < 0.05$ and 95%CI does not include 1. Heterogeneity among studies was assessed by calculating I^2 measure of inconsistency which was considered significant if $P < 0.10$ or $I^2 > 50\%$. If heterogeneity was statistically significant, a study elimination analysis was utilized to examine for heterogeneity when certain studies were excluded from the analysis. RevMan 5.1 was utilized for statistical analysis. Publication bias was assessed by funnel plots.

RESULTS

The initial search identified 242 articles and trials (Figure 1). Nine studies satisfied the inclusion criteria ($n = 2911$) with a mean age range from 53.0 to 59.6 years. Table 1 shows a summary of the details for each study including the low-volume and full-dose preparations. All studies used 2 L PEG with ascorbic acid *vs* 3 or 4 L PEG solutions.

Bowel preparations

Eight studies examined the number of satisfactory bowel

Table 1 Details of studies included in the meta-analysis

Author	Type of study	Blinding	Location	No. of patients	Low-volume bowel preparation	Full-dose bowel preparation	Jadad Score
Clark *et al*[27] 2007	RCT Abstract	Single	Not specified	294	2 L PEG with ascorbic acid	4 L PEG	1
Ell *et al*[24] 2008	RCT	Single	Germany	308	2 L PEG with ascorbic acid	4 L PEG	3
Lee *et al*[26] 2008	RCT Abstract	Single	Not specified	56	2 L PEG with ascorbic acid	4 L PEG	1
Corporaal *et al*[22] 2010	RCT	Single	Netherlands	307	2 L PEG with ascorbic acid	4 L PEG	2
Marmo *et al*[23] 2010	RCT	Single	Italy	433	2 L PEG with ascorbic acid	4 L PEG	3
Pontone *et al*[19] 2011	RCT	Single	Italy	130	2 L PEG with ascorbic acid	4 L PEG with Simethicone	3
Jansen *et al*[21] 2011	RCT	Single	Netherlands	370	2 L PEG with ascorbic acid +/- Simethicone	4 L PEG +/- Simethicone	3
González-Méndez *et al*[25] 2011	RCT Abstract	Single	Spain	681	2 L PEG with ascorbic acid + Bisacodyl	3 L PEG + Bisacodyl	1
Valiante *et al*[20] 2012	RCT	Single	Italy	332	2 L PEG with ascorbic acid	4 L PEG	3

PEG: Polyethylene glycol; RCT: Randomized controlled trial.

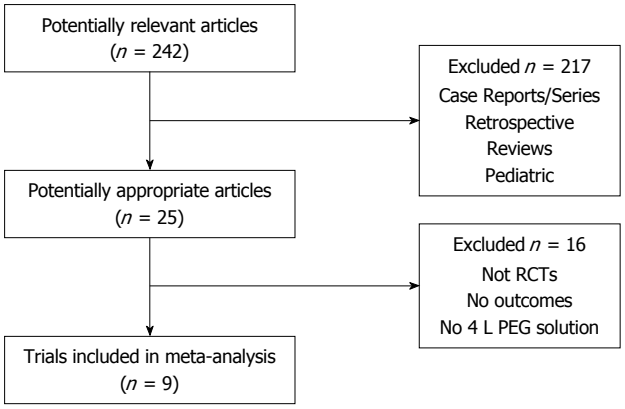

Figure 1 Article search results for this meta-analysis. PEG: Polyethylene glycol; RCT: Randomized controlled trial.

preparations (n = 2478)[19-22,24-27]. Among these 2478 patients, it was found that 1891 had a satisfactory bowel preparation with 950 in the 2 L PEG with ascorbic acid group and 941 in the full-dose PEG group. No statistically significant difference between the two groups was found when evaluating for satisfactory bowel preparation (OR 1.07, 95%CI: 0.86-1.33, P = 0.56). Figure 2 shows the Forest plot for satisfactory bowel preparations. No statistically significant heterogeneity was observed (I^2 = 42%, P = 0.10).

Five studies examined the number of poor bowel preparations (n = 1447)[19-22,24]. Figure 3 shows the Forest plot for these results. There was no significant difference for poor bowel preparation (OR 0.73, 95%CI: 0.48-1.11, P = 0.14) between the two groups. No significant heterogeneity was noted in the poor bowel preparation group (I^2 = 0%, P = 0.64).

Gastrointestinal side effects

Gastrointestinal side effects including abdominal pain[19-24] (n = 1880), nausea[19,20,22-24] (n = 1510), and vomiting[19,20,22-25] (n = 2191) were analyzed. No statistically significant difference was found for abdominal pain (OR 1.09, 95%CI: 0.81-1.48, P = 0.56) or vomiting (OR 0.99, 95%CI: 0.78-1.26, P = 0.95) (Table 2). A trend was noted for less nausea in the 2 L with ascorbic acid as compared to full-dose PEG; however, no statistical significance was reached (OR 0.70, 95%CI: 0.49-1.00, P = 0.05).

Table 2 Outcomes of side effects analyzed between low-volume polyethylene glycol with ascorbic acid and full-dose polyethylene glycol before colonoscopy

Side effect	OR	95%CI	*P*-value	Significance
Abdominal pain	1.09	0.81-1.48	0.56	NS
Nausea	0.70	0.49-1.00	0.05	NS
Vomiting	0.99	0.78-1.26	0.95	NS

NS: Not significant.

Publication bias

No statistically significant publication bias was noted (Figure 4).

DISCUSSION

Colonoscopy is a widely available and highly useful diagnostic tool for evaluating colonic and terminal ileal disease. Its success largely depends on an adequate bowel preparation to allow a thorough examination of the colonic and ileal mucosa. Various bowel preparations have been developed over the years under the premise that an ideal bowel preparation is one that is palatable to the patient, effective in cleansing quality, relatively small in volume, and tolerated well by patients with minimal adverse gastrointestinal symptoms.

One of the most commonly used bowel preparations has been 4 L of PEG solution. While effective, it requires the patient to consume a large amount of volume over a short period of time, resulting in some that are unable to tolerate the preparation. Due to this large volume, several recent studies, including a meta-analysis, have evaluated the effectiveness of administering the PEG solution in a split-dose with half given the evening before and half given the morning of the procedure[30]. While this study showed an improvement in bowel cleansing and decrease in some gastrointestinal side effects, patients still need to

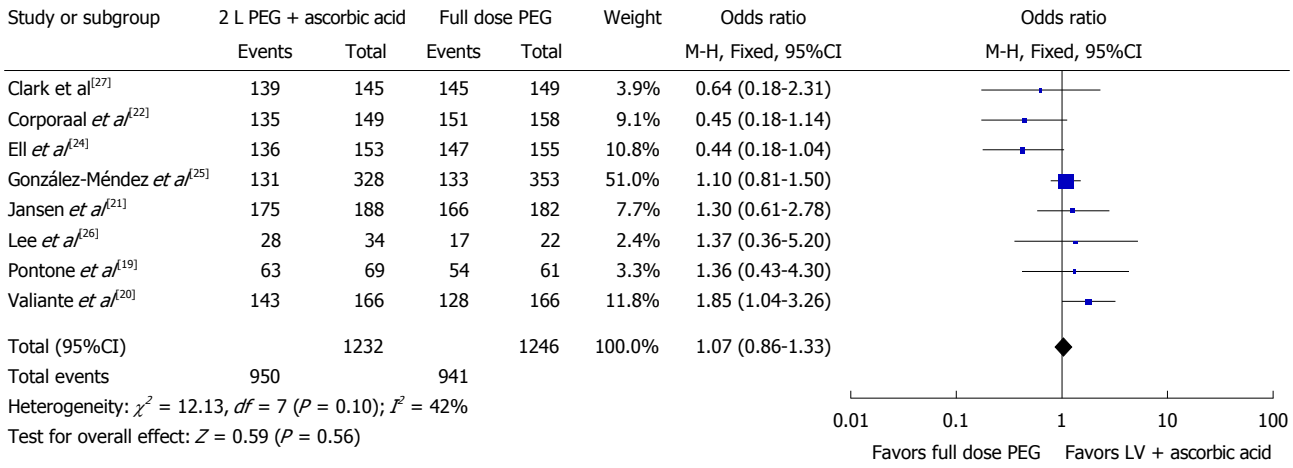

Figure 2 Forest plot for satisfactory bowel preparations between low-volume polyethylene glycol with ascorbic acid compared to full-dose polyethylene glycol. PEG: Polyethylene glycol.

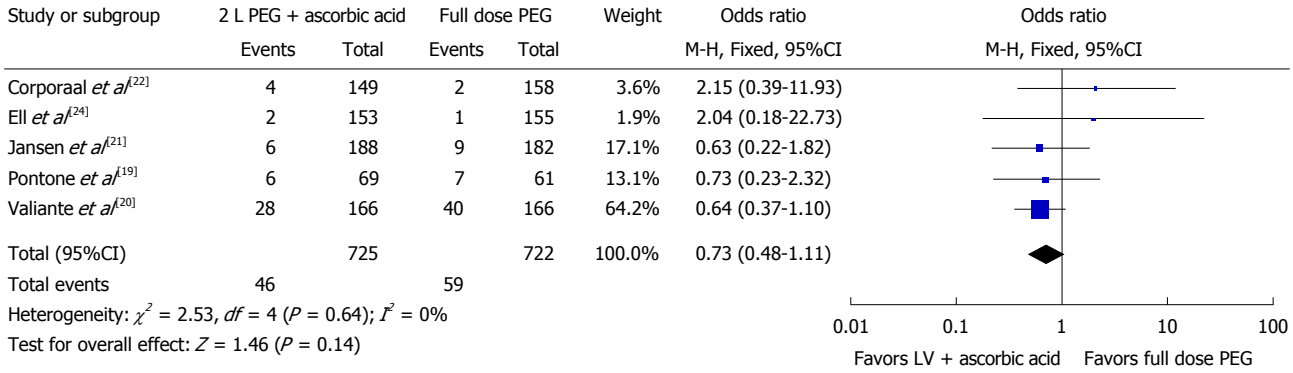

Figure 3 Forest plot for poor bowel preparations between low-volume polyethylene glycol with ascorbic acid compared to full-dose polyethylene glycol. PEG: Polyethylene glycol.

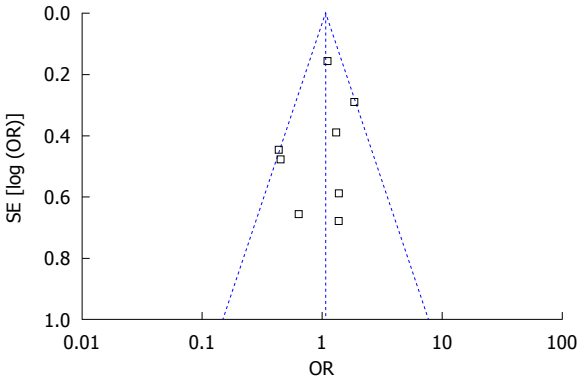

Figure 4 Funnel plot demonstrating no publication bias.

consume 4 L of PEG solution. Other studies have used lower-volume 2 L PEG solutions with various adjuncts including senna, bisacodyl, or magnesium citrate. These studies showed an improvement in tolerability but suggested a decrease in efficacy[16-18]. More recently, several studies have been conducted to evaluate the effectiveness and tolerability of a low-volume 2 L PEG solution with ascorbic acid as compared to full-dose 4 L PEG. These studies suggested that the reduced volume solution is effective in bowel cleansing but may not offer any advantages in reducing potential gastrointestinal side-effects.

Our meta-analysis was conducted to clarify the overall effects of a low-volume 2 L PEG solution with ascorbic acid compared to full-dose 4 L PEG solution. Only RCTs in adult patients were evaluated and used in this study. Based on our findings, low-volume PEG with ascorbic acid was equally effective in producing a satisfactory bowel preparation during colonoscopy, suggesting this to be a reasonable alternative to full-dose 4 L PEG solution with comparable bowel cleansing properties. However, patients receiving the low-volume 2 L PEG solution with ascorbic acid showed a similar pattern in gastrointestinal side effects including abdominal pain, nausea, and vomiting when compared to full-dose 4 L PEG solution, offering no overt advantage. One possible explanation for this is that patients receiving the 2 L PEG solution with ascorbic acid are required to consume an additional 500 mL of clear liquids after each 1 L of solution, totaling 3 L of liquid volume consumed during this preparation. One could argue that this still requires patients to ingest a moderate-to-large amount of fluid during a short period of time.

The strengths of our meta-analysis include the use of RCTs in various populations and end-points that are

significant to clinical practice. This also represents the first meta-analysis performed on this subject. However, a few limitations to this meta-analysis do exist. First, uniformity between the studies in using only 2 L PEG with ascorbic acid and full-dose PEG solution was not consistent among all studies. González-Méndez *et al*[25] used a 3 L PEG solution rather than the typical 4 L PEG solution. This could alter the results as patients ingested an equal volume of liquid (3 L) in both groups. However, if this study was eliminated, the overall results were similar (Satisfactory prep: OR 1.04, 95%CI: 0.75-1.43, P = 0.82). Additionally, a few studies utilized other adjuncts such as bisacodyl[25] and simethicone[19,21]. Given that simethicone is not a laxative, its addition in these studies likely had little impact on the quality of bowel cleansing. However, although bisacodyl is a laxative, it was given to both arms of the study, negating its overall effect. Second, a limited number of studies were used in this meta-analysis; however, all studies to-date were included in this meta-analysis using an extensive search protocol. Third, the quality of the studies was not ideal. As in most bowel preparation studies, it is very difficult to blind the patient. Therefore, these RCT's were single-blinded to the colonoscopist, which is the optimal format for these studies. Also, three of the studies were abstracts with no data regarding method of randomization or blinding, leading to a lower Jadad score. However, these abstract studies were single-blinded randomized trials and due to word limits on abstracts, may not have presented their randomization and blinding techniques, which does not make them any less quality than other bowel prep studies. Finally, slightly different bowel prep rating systems were utilized among studies. However, all studies specifically defined satisfactory or unsatisfactory bowel preparations based upon their specific scale.

In conclusion, our meta-analysis found that a low-volume 2 L PEG solution with ascorbic acid administered for bowel preparation prior to colonoscopy provided equal bowel cleansing when compared to a full-dose 4 L PEG solution. However, the reduced volume of the 2 L PEG solution with ascorbic acid did not provide any benefit when comparing gastrointestinal side-effects including abdominal pain, nausea, and vomiting. Therefore, the low-volume 2 L PEG solution with ascorbic acid can be considered as an appropriate and equally effective bowel preparation prior to colonoscopy but does not appear to offer any advantage over the traditional 4 L PEG solution. Further studies are required to compare the 2 L with ascorbic acid to the newer 4 L split-dose bowel preparation.

COMMENTS

Background

Colorectal cancer (CRC) is a major cause of cancer-related deaths worldwide. Colonoscopy has become a widely available screening test for both preventing and detecting CRC. However, colonoscopy requires an adequate bowel preparation for complete visualization which may induce unwanted side effects and patient discomfort.

Research frontiers

Several studies have compared the standard bowel preparation of 4 L polyethylene glycol (PEG) to a 2 L PEG solution with ascorbic acid. This study is a meta-analysis comparing the above mentioned bowel preparations with regards to adequacy of the bowel preparation as well as patient side-effects during ingestion of the bowel preparation.

Innovations and breakthroughs

This is the first meta-analysis comparing 2 L PEG solution with ascorbic acid to 4 L PEG solution. We found that the 2 L PEG solution with ascorbic acid provided equal bowel cleansing when compared to a full-dose 4 L PEG solution. However, the reduced volume of the 2 L PEG solution with ascorbic acid did not provide any benefit when comparing gastrointestinal side-effects including abdominal pain, nausea, and vomiting.

Applications

The low-volume 2 L PEG solution with ascorbic acid can be considered as an appropriate and equally effective bowel preparation prior to colonoscopy but does not appear to offer any advantage over the traditional 4 L PEG solution.

Terminology

PEG is a common bowel cleansing solution that was first introduced in 1980. Standard bowel preparation using PEG typically involves ingestion of 4 L of solution prior to colonoscopy.

Peer review

This is an interesting study, and a well written paper.

REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]

2 **Rex DK**, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM. American College of Gastroenterology guidelines for colorectal cancer screening 2009 [corrected]. *Am J Gastroenterol* 2009; **104**: 739-750 [PMID: 19240699 DOI: 10.1038/ajg.2009.104]

3 **Giardiello FM**, Gurbuz AK, Bayless TM, Goodman SN, Yardley JH. Colorectal cancer in ulcerative colitis: survival in patients with and without colorectal cancer symptoms. *Inflamm Bowel Dis* 1996; **2**: 6-10 [PMID: 23282450]

4 **Laine L**, Shah A. Randomized trial of urgent vs. elective colonoscopy in patients hospitalized with lower GI bleeding. *Am J Gastroenterol* 2010; **105**: 2636-2641; quiz 2642 [PMID: 20648004 DOI: 10.1038/ajg.2010.277]

5 **Schusselé Filliettaz S**, Juillerat P, Burnand B, Arditi C, Windsor A, Beglinger C, Dubois RW, Peytremann-Bridevaux I, Pittet V, Gonvers JJ, Froehlich F, Vader JP. Appropriateness of colonoscopy in Europe (EPAGE II). Chronic diarrhea and known inflammatory bowel disease. *Endoscopy* 2009; **41**: 218-226 [PMID: 19280533 DOI: 10.1055/s-0028-1119627]

6 **Temmerman F**, Baert F. Collagenous and lymphocytic colitis: systematic review and update of the literature. *Dig Dis* 2009; **27** Suppl 1: 137-145 [PMID: 20203510 DOI: 10.1159/000268134]

7 **Parente F**, Marino B, Crosta C. Bowel preparation before colonoscopy in the era of mass screening for colo-rectal cancer: a practical approach. *Dig Liver Dis* 2009; **41**: 87-95 [PMID: 18676211 DOI: 10.1016/j.dld.2008.06.005]

8 **Swaroop VS**, Larson MV. Colonoscopy as a screening test for colorectal cancer in average-risk individuals. *Mayo Clin Proc* 2002; **77**: 951-956 [PMID: 12233928]

9 **Lichtenstein G**. Bowel preparations for colonoscopy: a review. *Am J Health Syst Pharm* 2009; **66**: 27-37 [PMID: 19106342 DOI: 10.2146/ajhp080084]

10 **Barkun A**, Chiba N, Enns R, Marcon M, Natsheh S, Pham C, Sadowski D, Vanner S. Commonly used preparations for colonoscopy: efficacy, tolerability, and safety--a Canadian Association of Gastroenterology position paper. *Can J Gastroenterol* 2006; **20**: 699-710 [PMID: 17111052]

11 **Wexner SD**, Beck DE, Baron TH, Fanelli RD, Hyman N, Shen B, Wasco KE. A consensus document on bowel preparation before colonoscopy: prepared by a task force from the

American Society of Colon and Rectal Surgeons (ASCRS), the American Society for Gastrointestinal Endoscopy (ASGE), and the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES). *Gastrointest Endosc* 2006; **63**: 894-909 [PMID: 16733101 DOI: 10.1016/j.gie.2006.03.918]

12 **Khalid-de Bakker CA**, Jonkers DM, Hameeteman W, de Ridder RJ, Masclee AA, Stockbrügger RW. Opportunistic screening of hospital staff using primary colonoscopy: participation, discomfort and willingness to repeat the procedure. *Digestion* 2011; **84**: 281-288 [PMID: 22041853 DOI: 10.1159/000327383]

13 **Jung B**, Lannerstad O, Påhlman L, Arodell M, Unosson M, Nilsson E. Preoperative mechanical preparation of the colon: the patient's experience. *BMC Surg* 2007; **7**: 5 [PMID: 17480223 DOI: 10.1186/1471-2482-7-5]

14 **DiPalma JA**, Brady CE, Pierson WP. Colon cleansing: acceptance by older patients. *Am J Gastroenterol* 1986; **81**: 652-655 [PMID: 3740024]

15 **Davis GR**, Santa Ana CA, Morawski SG, Fordtran JS. Development of a lavage solution associated with minimal water and electrolyte absorption or secretion. *Gastroenterology* 1980; **78**: 991-995 [PMID: 7380204]

16 **Hookey LC**, Depew WT, Vanner SJ. Combined low volume polyethylene glycol solution plus stimulant laxatives versus standard volume polyethylene glycol solution: a prospective, randomized study of colon cleansing before colonoscopy. *Can J Gastroenterol* 2006; **20**: 101-105 [PMID: 16482236]

17 **Sharma VK**, Chockalingham SK, Ugheoke EA, Kapur A, Ling PH, Vasudeva R, Howden CW. Prospective, randomized, controlled comparison of the use of polyethylene glycol electrolyte lavage solution in four-liter versus two-liter volumes and pretreatment with either magnesium citrate or bisacodyl for colonoscopy preparation. *Gastrointest Endosc* 1998; **47**: 167-171 [PMID: 9512283 DOI: 10.1016/S0016-5107(98)70351-7]

18 **DiPalma JA**, Wolff BG, Meagher A, Cleveland Mv. Comparison of reduced volume versus four liters sulfate-free electrolyte lavage solutions for colonoscopy colon cleansing. *Am J Gastroenterol* 2003; **98**: 2187-2191 [PMID: 14572566 DOI: 10.1111/j.1572-0241.2003.07690.x]

19 **Pontone S**, Angelini R, Standoli M, Patrizi G, Culasso F, Pontone P, Redler A. Low-volume plus ascorbic acid vs high-volume plus simethicone bowel preparation before colonoscopy. *World J Gastroenterol* 2011; **17**: 4689-4695 [PMID: 22180711 DOI: 10.3748/wjg.v17.i42.4689]

20 **Valiante F**, Pontone S, Hassan C, Bellumat A, De Bona M, Zullo A, de Francesco V, De Boni M. A randomized controlled trial evaluating a new 2-L PEG solution plus ascorbic acid vs 4-L PEG for bowel cleansing prior to colonoscopy. *Dig Liver Dis* 2012; **44**: 224-227 [PMID: 22119219 DOI: 10.1016/j.dld.2011.10.007]

21 **Jansen SV**, Goedhard JG, Winkens B, van Deursen CT. Preparation before colonoscopy: a randomized controlled trial comparing different regimes. *Eur J Gastroenterol Hepatol* 2011; **23**: 897-902 [PMID: 21900786 DOI: 10.1097/MEG.0b013e32834a3444]

22 **Corporaal S**, Kleibeuker JH, Koornstra JJ. Low-volume PEG plus ascorbic acid versus high-volume PEG as bowel preparation for colonoscopy. *Scand J Gastroenterol* 2010; **45**: 1380-1386 [PMID: 20602568 DOI: 10.3109/00365521003734158]

23 **Marmo R**, Rotondano G, Riccio G, Marone A, Bianco MA, Stroppa I, Caruso A, Pandolfo N, Sansone S, Gregorio E, D'Alvano G, Procaccio N, Capo P, Marmo C, Cipolletta L. Effective bowel cleansing before colonoscopy: a randomized study of split-dosage versus non-split dosage regimens of high-volume versus low-volume polyethylene glycol solutions. *Gastrointest Endosc* 2010; **72**: 313-320 [PMID: 20561621 DOI: 10.1016/j.gie.2010.02.048]

24 **Ell C**, Fischbach W, Bronisch HJ, Dertinger S, Layer P, Rünzi M, Schneider T, Kachel G, Grüger J, Köllinger M, Nagell W, Goerg KJ, Wanitschke R, Gruss HJ. Randomized trial of low-volume PEG solution versus standard PEG + electrolytes for bowel cleansing before colonoscopy. *Am J Gastroenterol* 2008; **103**: 883-893 [PMID: 18190651 DOI: 10.1111/j.1572-0241.2007.01708.x]

25 **González-Méndez Y**, Alarcón-Fernández O, Romero-García R, Adrián-De-Ganzo Z, Alonso-Abreu I, Carrillo-Palau M, Quintero E, Jiménez-Sosa A. Comparison of colon cleansing with two liters of polyethylen glycol-ascorbic acid versus three liters of polyethylen glycol-electrolyte: A prospective and randomized study [abstract]. *Gastrointest Endosc* 2011; **73**: AB424 [DOI: 10.1016/j.gie.2011.03.976]

26 **Lee BC**, Moyes DA, Mcloughlin JC, Lim PL. The efficacy, acceptability and safety of the new 2L polyethylene glycol + electrolytes + ascorbic acid (PEG + E + ASC) vs the 4L polyethylene glycol 3350 + electrolytes (PEG + E) in patients undergoing elective colonoscopies in a UK teaching hospital [abstract]. *Gastrointest Endosc* 2008; **67**: AB290-AB291 [DOI: 10.1016/j.gie.2008.03.836]

27 **Clark L**, Gruss HJ, Kloess HR, Dugue C, Geraint M, Halphen M, Marsh S. Better efficacy of a new 2 litre bowel cleansing preparation in the ascending colon [abstract]. *Gastrointest Endosc* 2007; **65**: AB262 [DOI: 10.1016/j.gie.2007.03.617]

28 **Jadad AR**, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**: 1-12 [PMID: 8721797 DOI: 10.1016/0197-2456(95)00134-4]

29 **Jüni P**, Altman DG, Egger M. Systematic reviews in health care: Assessing the quality of controlled clinical trials. *BMJ* 2001; **323**: 42-46 [PMID: 11440947 DOI: 10.1136/bmj.323.7303.42]

30 **Kilgore TW**, Abdinoor AA, Szary NM, Schowengerdt SW, Yust JB, Choudhary A, Matteson ML, Puli SR, Marshall JB, Bechtold ML. Bowel preparation with split-dose polyethylene glycol before colonoscopy: a meta-analysis of randomized controlled trials. *Gastrointest Endosc* 2011; **73**: 1240-1245 [PMID: 21628016 DOI: 10.1016/j.gie.2011.02.007]

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META-ANALYSIS

Smoking increases risk of tooth loss: A meta-analysis of the literature

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Abstract

AIM: To quantitatively evaluate the impact of smoking on tooth loss.

METHODS: We performed a PubMed search to identify published articles that investigated the risk of tooth loss by smoking, from which RRs and their variance with characteristics of each study were extracted. The random-effects models were used to derive a pooled effect across studies. Potential sources of heterogeneity on the characteristics of the study and their influence on the pooled effect size were investigated using meta-regression models.

RESULTS: We identified 24 studies containing a total of 95973 participants for analysis. The pooled RR of ever-smokers compared with never- smokers was 1.73 (95%CI: 1.60-1.86, $P < 0.001$). In meta-regression analysis, only the mean age of participants alone was identified as a statistically significant source of heterogeneity. The effect of smoking on tooth loss was stronger when the mean age of study participants was higher, indicating possible enhancement of tooth loss due to aging by smoking. RR was significantly lower in former smokers (1.49, 95%CI: 1.32-1.69, $P < 0.001$) than in current smokers (2.10, 95%CI: 1.87-2.35, $P < 0.001$), indicating the substantial benefit of smoking cessation for reducing the risk of tooth loss.

CONCLUSION: Smoking is an independent risk factor for tooth loss regardless of many other confounders. Smoking cessation may attenuate this effect.

Key words: Meta-analysis; Oral health; Relative risk; Smoking; Tooth loss

Core tip: Smoking is known to be a major cause of tooth loss. However, it has never been known how it quantitatively attributes to tooth loss or whether smoking cessation counteracts or not. This study clarified that ever smoking increases risk of tooth loss by 73%. In addition, smoking cessation substantially attenuates this effect.

Sato F, Sawamura M, Ojima M, Tanaka K, Hanioka T, Tanaka H, Matsuo K. Smoking increases risk of tooth loss: A meta-analysis

of the literature. *World J Meta-Anal* 2013; 1(1): 16-26 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i1/16.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i1.16

INTRODUCTION

The World Health Organization Global Oral Health Program works to increase awareness of oral health worldwide as an important component of general health and quality of life[1]. A number of studies have investigated the association between tooth loss and cardiovascular diseases, including stroke, atherosclerosis and hypertension[2-8]. Several reviews outlined a possible role for tooth loss in carcinogenesis, independent of other known risk factors[9,10]. Tooth loss is one of the main impediments to oral health; and by affecting the patient's ability to chew and thus altering food choices and the digestive process, may lead to malnutrition[11,12]. The impact of tooth loss can be even more severe, impairing taste, phonetics, and aesthetics, often resulting in limited social and personal interaction[13,14]. A systematic review provided fairly strong evidence that tooth loss is associated with the impairment of oral health-related quality of life[15].

The etiology of tooth loss is complex, and includes factors such as age; sex; body mass index; physical activity; systemic disease, such as osteoporosis and diabetes; socioeconomic status (SES); and oral hygiene behavior[16-21]. Smoking is considered an important risk factor for tooth loss[16,18,19,22-26]. Although numerous studies have consistently reported a positive association, attempts to quantify the association have been hampered by their variation in background factors, such as country of the study, study design, age of participants, sex, and oral hygiene behavior.

The present study aims to: (1) confirm the association between smoking and tooth loss, and to quantify the impact systematically; (2) to confirm the difference in the impact of smoking on tooth loss between former and current smokers; and (3) to investigate the difference in the impact of smoking on tooth loss by the factors above. To our knowledge, this study is the first meta-analysis to quantify the impact of smoking on tooth loss.

MATERIALS AND METHODS

Search strategy

The initial literature search was conducted through PubMed using the free text search term: (tooth loss OR missing tooth OR oral health OR oral hygiene) AND (smoking OR smoke OR cigarette), with publication period updated to July 2010.

We selected candidate studies based on the following inclusion criteria: original article published in English; and the availability of RRs estimates of smoking for tooth loss in the article, namely hazard ratio, risk ratio, or odds ratio, with the reference group consisting of never smokers, and with adjustment for age at least, and their 95%CIs. Two investigators (FS and MS) independently reviewed all potentially relevant articles, and disagreements were resolved by discussion. The reference lists of the studies identified through this process were also checked.

Data extraction

Characteristics extracted from the articles included name of the first author, year of publication, country of study, study design (cohort or cross-sectional study), base population, sex distribution, number of participants, mean age of study population, measure of association (hazard ratio, risk ratio, or odds ratio), point estimate and its 95%CI of RR, adjustment for SES (yes or no), adjustment for behavior associated with oral health (yes or no), and definition of the number of teeth lost.

Data synthesis

For inclusion in quantitative analysis, studies had to provide sufficient data to allow calculation of an effect-size measure and its corresponding measure of variability. Because we extracted multiple estimates from several studies (*e.g.*, using pack-year units or stratified analysis), we pre-pooled RRs to derive one overall RR for each study using fixed-effects estimates weighted by the inverse of their variance as the RR for ever-smokers relative to never-smokers. All analyses were performed on the natural log scale. Because of the widely different methodological approaches used to examine the relationship in the individual studies, we used the random-effects models of DerSimonian-Laird[27] to derive a pooled effect across studies, in which the between-study variance was estimated in addition to the specified within-variance component. We investigated potential sources of heterogeneity on the characteristics of the study and their influence on the pooled effect size using meta-regression models. We examined heterogeneity using Cochrane's Q-test and the I^2 statistic[28]. I^2 can be interpreted as the proportion of the total variation in the estimated slopes for each study due to heterogeneity between studies. Variables considered as potential sources of heterogeneity were the country in which the study was conducted [United States (reference), Japan, Nordic, and others as dummy variables], study design (cohort or cross-sectional), base population (general population or other), sex included in the study (male, female, or both, as dummy variables), mean age of the study population (continuous), adjustment by SES, adjustment by behavior associated with oral health, and definition of the number of teeth lost (continuous).

Publication bias was assessed by a funnel plot with the fitted line corresponding to the regression test for funnel-plot asymmetry proposed by Egger *et al*[29].

All analyses were conducted using the *metan* and *metareg* commands in STATA ver 10.1 (Stata Corporation, College Station, Texas, USA) and were two-sided. Tests were considered statistically significant when the P value were less than 0.05, except in meta-regression analysis, for which we defined a threshold P value of less than 0.1.

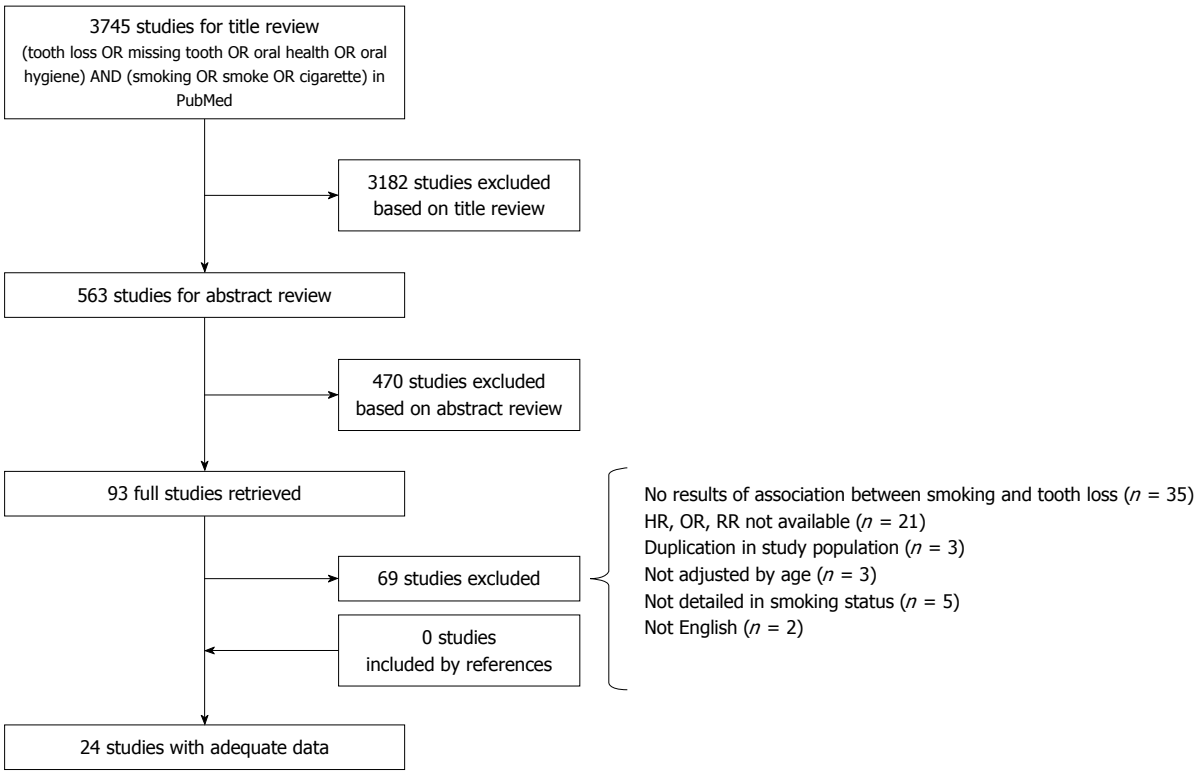

Figure 1 Selection of literature.

RESULTS

Search results

A total of 3745 potentially relevant reports were identified. Of these, 93 full papers were obtained based on title and abstract review (Figure 1), of which 24 with a total of 95973 participants were identified as having sufficient data for inclusion[16-26,30-42].

Table 1 shows the baseline characteristics of participants from each study. By country, seven papers were from the USA, six from Japan, three each from Brazil and Finland, and one each from Sweden, Norway, Germany, Italy, and Australia. Of these, five were cohort studies, with a mean follow-up of 9.4 years; 16 were conducted in general populations; 16 were conducted in both sexes; 15 investigated the risk of tooth loss in current and former *vs* never- smokers; nine investigated the risk of tooth loss in ever- *vs* never-smokers; 15 were adjusted by SES; and 15 were adjusted by behavior associated with oral health. The studies varied in study size (range, 166-8409 for cross-sectional studies, 693-43112 for cohort studies), mean age of the study population (21.5-81.0 years), and definition of the number of teeth lost as a dependent variable. All studies used multivariate analysis to calculate the RR of tooth loss by smoking.

Association between smoking history and risk of tooth loss

Results for the meta-analysis of RRs of tooth loss in ever- *vs* never-smokers are shown in Figure 2. A forest plot of the random-effects model analysis showed that four of the five earliest studies[16-18,30,31] tended to show a higher RR of tooth loss in ever-smokers than those published later. Pooled RR as estimated by the random-effects model was 1.73 (95%CI: 1.60-1.86). Significant heterogeneity was seen between studies, with a P value of < 0.001 and I^2 of 67.4%.

Modifiable factors in smoking history and risk of tooth loss

We used meta-regression analysis to investigate sources of heterogeneity for the relationship between smoking and tooth loss (Table 2). In univariate meta-regression analysis, mean age of the study population ($P = 0.009$) and definition of the number of teeth lost ($P = 0.040$) were identified as potential sources of heterogeneity. Figure 3A and B show the results of meta-analyses sorted by mean age of study population and definition of the number of teeth lost. In multivariate meta-regression with significant modifiers detected by these two variables, mean age of the study population remained as the potentially strongest source of heterogeneity ($P = 0.030$).

Publication bias

We also assessed potential publication bias in selected studies. A funnel plot (Figure 4) shows the distribution of log-transformed RR and standard error in each study, with the fitted line corresponding to the regression test for funnel-plot asymmetry (solid line). Studies with large standard errors with weaker associations seemed less reported; however, the association remained significant even after exclusion of studies with large standard errors greater

Table 1 Baseline characteristics of patients of the 24 included studies

Author (yr)	Country	Study design	Base population	Sex	*n*	Mean age (yr) of subjects	Measure of association	Pattern of comparison (*vs* never smokers)	Relative risk	Adjustment for socioeconomic status	Adjustment for behavior associated with oral health[3]	Definition, No. of tooth loss
Eklund *et al*[16] (1994)	United States	Cohort	General population	M/F	2207	42.0	RR	Ever	1.88 (1.04-3.38)[1]	Yes	Yes	Incidence of tooth loss
Norlén *et al*[18] (1996)	Sweden	Cross-sectional	General population	M	483	68.0	OR	Former	2.60 (1.34-5.03)	Yes	Yes	> 16 loss
								Current	3.02 (1.50-6.07)			
								Ever	2.79 (1.73-4.52)[2]			
Slade *et al*[30] (1997)	Australia	Cohort	General population	M/F	693	73.0	RR	Former	2.55 (1.48-4.40)	No	Yes	Incidence of tooth loss
								Current	2.06 (0.92-4.62)			
								Ever	2.39 (1.52-3.74)[2]			
Suominen-Taipale *et al*[31] (1999)	Finland	Cross-sectional	General population	M/F	213	40.0	OR	Ever	1.4 (1.0-2.0)	Yes	No	28 loss
Xie *et al*[17] (1999)	Finland	Cross-sectional	General population	M/F	293	81.0	OR	Ever	3.12 (1.56-6.23)	No	No	28 loss
Yoshida *et al*[33] (2001)	Japan	Cross-sectional	Petroleum chemical plant employees	M	2015	39.5	OR	Former	1.27 (0.89-1.81)	No	Yes	> 1 loss
								Current	1.54 (1.20-1.96)			
								Ever	1.45 (1.18-1.77)[2]			
Randolph *et al*[32] (2001)	United States	Cross-sectional	General population	M/F	3050	74.1	OR	Former	1.26 (1.04-1.54)	Yes	No	> 14 loss
								Current	1.69 (1.31-2.20)			
								Ever	1.40 (1.20-1.63)[2]			
Ylöstalo *et al*[19] (2004)	Finland	Cross-sectional	General population	M/F	8409	31.0	OR	Ever	1.73 (1.39-2.15)[2]	Yes	Yes	> 6 loss
Cunha-Cruz *et al*[22] (2004)	Brazil	Cross-sectional	University employees	M/F	3840	40.0	OR	Ever	1.62 (1.35-1.96)	Yes	Yes	> 26 loss
Klein *et al*[34] (2004)	United States	Cross-sectional	General population	M/F	2794	65.0	OR	Former	1.57 (1.25-1.98)	Yes	No	> 1 loss
								Current	4.04 (2.52-6.49)			
								Ever	1.88 (1.53-2.31)[2]			
Tanaka *et al*[35] (2005)	Japan	Cross-sectional	Hospital	F	1002	29.8	OR	Former	1.42 (0.91-2.20)	Yes	No	> 1 loss
								Current	1.56 (1.18-2.06)[2]			
								Ever	1.52 (1.20-1.93)[2]			
Susin *et al*[20] (2005)	Brazil	Cross-sectional	General population	M/F	974	48.7	OR	Ever	1.50 (1.18-1.90)[2]	No	No	> 7 loss
Susin *et al*[37] (2006)	Brazil	Cross-sectional	General population	M/F	612	21.5	OR	Ever	1.30 (1.02-1.66)[2]	Yes	No	> 1 loss
Okamoto *et al*[36] (2006)	Japan	Cross-sectional	Hospital	M	1332	43.5	OR	Former	1.11 (0.68-1.85)	No	No	> 1 loss
								Current	1.59 (1.21-2.08)[2]			
								Ever	1.46 (1.15-1.85)[2]			
Krall *et al*[23] (2006)	United States	Cohort	People who received dental care	M	789	49.0	HR	Former	1.3 (0.9-1.7)	Yes	Yes	Incidence of tooth loss
								Current	2.1 (1.5-3.1)			
								Ever	1.7 (1.3-2.2)[2]			
Ojima *et al*[40] (2007)	Japan	Cross-sectional	General population	M/F	1314	30.0	OR					
				Total				Former	0.80 (0.45-1.43)[2]	No	Yes	> 1 loss
								Current	1.91 (1.41-2.59)[2]			
								Ever	1.58 (1.21-2.07)[2]			
				M				Former	1.25 (0.55-2.86)			
								Current	2.21 (1.40-3.50)			
								Ever	1.93 (1.30-2.88)[2]			
				F				Former	0.52 (0.23-1.18)			
								Current	1.70 (1.13-2.55)			
								Ever	1.34 (0.93-1.93)[2]			

Mundt *et al*[24] (2007)	Germany	Cross-sectional	General population	M/F	2501	49.5	OR	Former	1.71 (1.27-2.30)	Yes	Yes	> 26 loss
								Current	2.58 (2.03-3.27)			
								Ever	2.19 (1.82-2.64)[2]			
Dietrich *et al*[25] (2007)	United States	Cohort	Health professional	M	43112	56.0	HR	Former	1.57 (1.53-1.62)[2]	Yes	Yes	Incidence of tooth loss
								Current	2.25 (2.14-2.37)[2]			
								Ever	1.75 (1.69-1.80)[2]			
Hanioka *et al*[38] (2007)	Japan	Cross-sectional	General population	M/F	3999	60.0	OR					
				Total				Former	1.18 (0.87-1.59)	No	Yes	> 20 loss
								Current	2.19 (1.71-2.80)			
								Ever	1.70 (1.40-2.05)[2]			
				M				Former	1.29 (0.92-1.80)			
								Current	2.22 (1.61-3.06)			
								Ever	1.72 (1.36-2.17)[2]			
				F				Former	0.86 (0.46-1.60)			
								Current	2.14 (1.45-3.15)			
								Ever	1.66 (1.19-2.31)[2]			
Musacchio *et al*[39] (2007)	Italy	Cross-sectional	General population	M	1226	76.8	OR	Former	3.42 (2.42-4.82)	Yes	No	28 loss
								Current	4.01 (2.59-6.20)			
								Ever	3.64 (2.77-4.77)[2]			
Haugejorden *et al*[26] (2008)	Norway	Cross-sectional	General population	M/F	1092	47.9	OR	Ever	2.2 (1.3-3.7)	Yes	Yes	> 20 loss
Cunha-Cruz *et al*[41] (2008)	United States	Cohort	People who received dental care	M/F	12631	51.0	RR	Former	1.3 (1.1-1.4)	No	Yes	Incidence of tooth loss
								Current	2.3 (2.0,2.6)			
								Ever	1.8 (1.6-2.0)[2]			
Moedano *et al*[21] (2009)	United States	Cross-sectional	Hospital	M/F	166	69.1	OR	Ever	1.19 (0.49-2.87)	Yes	No	> 10 loss
Yanagisawa *et al*[42] (2010)	Japan	Cross-sectional	General population	M	1088	59.6	OR	Former	1.35 (0.94-1.94)	No	Yes	> 8 loss
								Current	1.67 (1.12-2.50)			
								Ever	1.49 (1.14-1.94)[2]			

[1]Relative risk was calculated from coefficient and standard error; [2]Within-study summary estimate by meta-analysis with fixed effect model; [3]Behaviors associated with oral health include tooth brushing frequencies, existence of periodontal disease at the baseline survey, use of floss, frequency of dental clinic visit and reason for visit and occupation (dentist or not), use of interdental brush, self-check of teeth and gum a using mirror, and experience of tooth brushing instruction. M: Male; F: Female.

than 0.2 (Figure 5). Egger's test also excluded the possibility of publication bias in estimating summary statistics ($P = 0.968$).

Difference in risk of tooth loss between former and current smokers

The potential difference in tooth loss events between former and current smokers was examined by stratified analysis (Figure 6). Nine studies were excluded from analysis because they did not assess the risk in former and current smokers separately. The meta-analysis revealed summary estimates of 1.49 (95%CI: 1.32-1.69) and 2.10 (95%CI: 1.87-2.35) for former and current smokers, respectively, indicating that former smokers have a significantly lower probability of tooth loss than current smokers.

DISCUSSION

This study is the first meta-analysis of the impact of smoking on tooth loss, and includes the difference in impact between former and current smokers. We found an approximately 70% greater risk of tooth loss in ever- than never-smokers. Moreover, we found that former smokers had a statistically significantly lower risk of tooth loss than current smokers, with current smokers showing a 110% increase in risk compared with 49% in former smokers. We also evaluated potential sources of heterogeneity by factors thought

Table 2 Source of heterogeneity by meta-regression analysis

Factors	Univariate			Multivariate		
	Coefficient	SE	*P* value	Coefficient	SE	*P* value
Published year	-0.0063553	0.01429	0.661	-	-	-
Country (*vs* United States)						
Japan	-0.0922341	0.11903	0.447	-	-	-
Finland, Norway, Sweden	0.1485642	0.14826	0.328	-	-	-
Other countries	0.1828302	0.12939	0.173	-	-	-
Study design (cohort *vs* cross-sectional)	0.0667818	0.12342	0.594	-	-	-
Base Population of Study (general population *vs* others)	-0.0761659	0.10297	0.467			
Sex (male *vs* female)						
Male	0.0739773	0.11212	0.517	-	-	-
Female	-0.1176073	0.24732	0.639	-	-	-
Mean age of study population	0.0080805	0.00284	0.009	0.0067276	0.00288	0.030
Adjustment for socioeconomic status (Yes *vs* No)	-0.0723939	0.10375	0.493	-	-	-
Adjustment for behavior associated with oral health (Yes *vs* No)	-0.0054823	0.10733	0.960	-	-	-
Definition number of tooth loss in the study (range: 1-28)	0.0093892	0.00430	0.040	0.0065434	0.00414	0.129

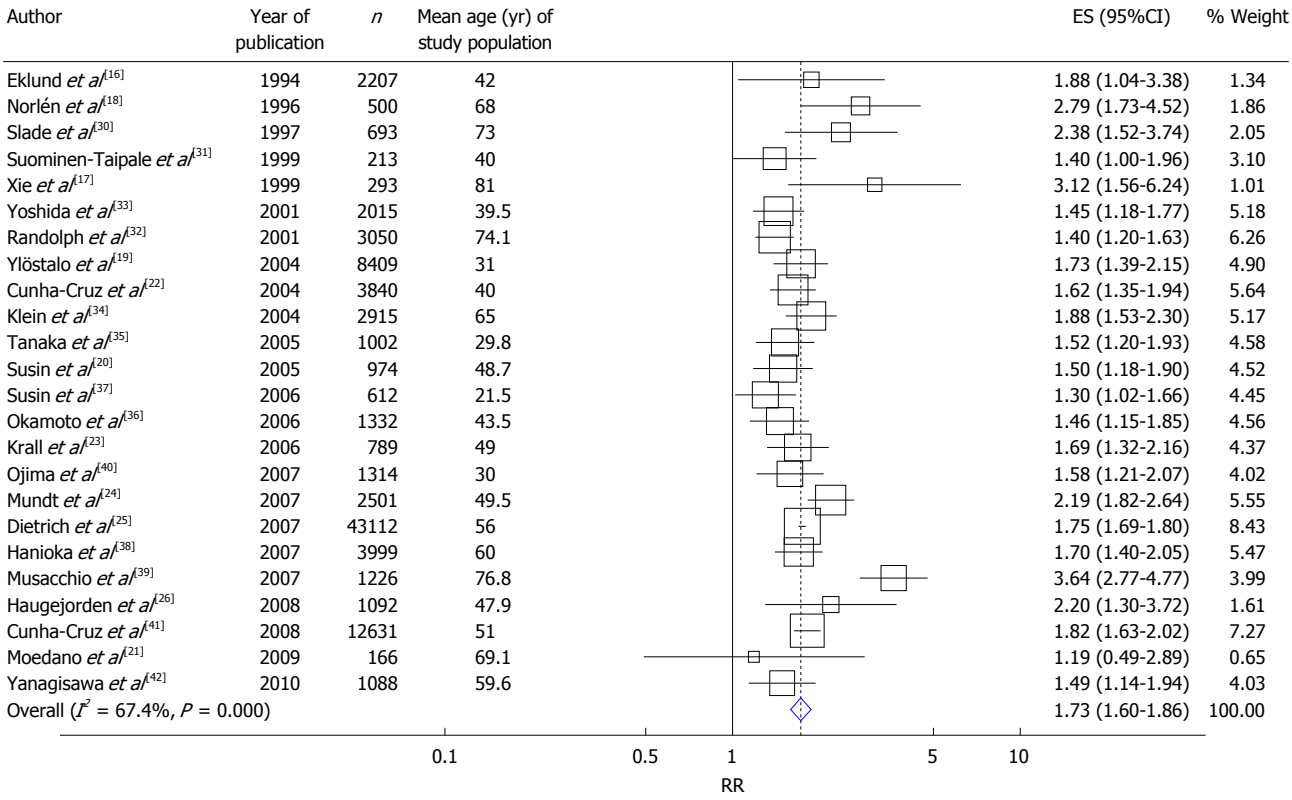

Figure 2 Forest plots of relative risk. The size of the squares corresponds to the weight of the study in the meta-analysis. Combined relative risk was calculated using the random-effects model. Weights are from random effects analysis.

to influence the effect of smoking on tooth loss. Results showed no statistically significant heterogeneity by country (included in the present study), study design, sex, oral health behavior, or SES except age. Although the risk of tooth loss by smoking showed heterogeneity by participant age, RRs of all studies were significantly higher in ever-smokers than in never-smokers, except for one study, which had the smallest number of participants of all studies analyzed[21].

Several mechanisms have been hypothesized to explain the association between smoking and tooth loss. Systemic effects of smoking include dysfunction of gingival fibroblasts, a decrease in microcirculatory function and immune system deficiency *via* effects of chemicals included in tobacco smoke[10,43]. Bacterial organisms in periodontal region are reported to contribute to tissue destruction among smokers[44-46]. These lines of evidences are consistent with findings in this study and are suggestive of importance of implementation of smoking cessation in the dental field[47].

We speculate that several factors might explain why the effect of smoking on tooth loss was modified by age.

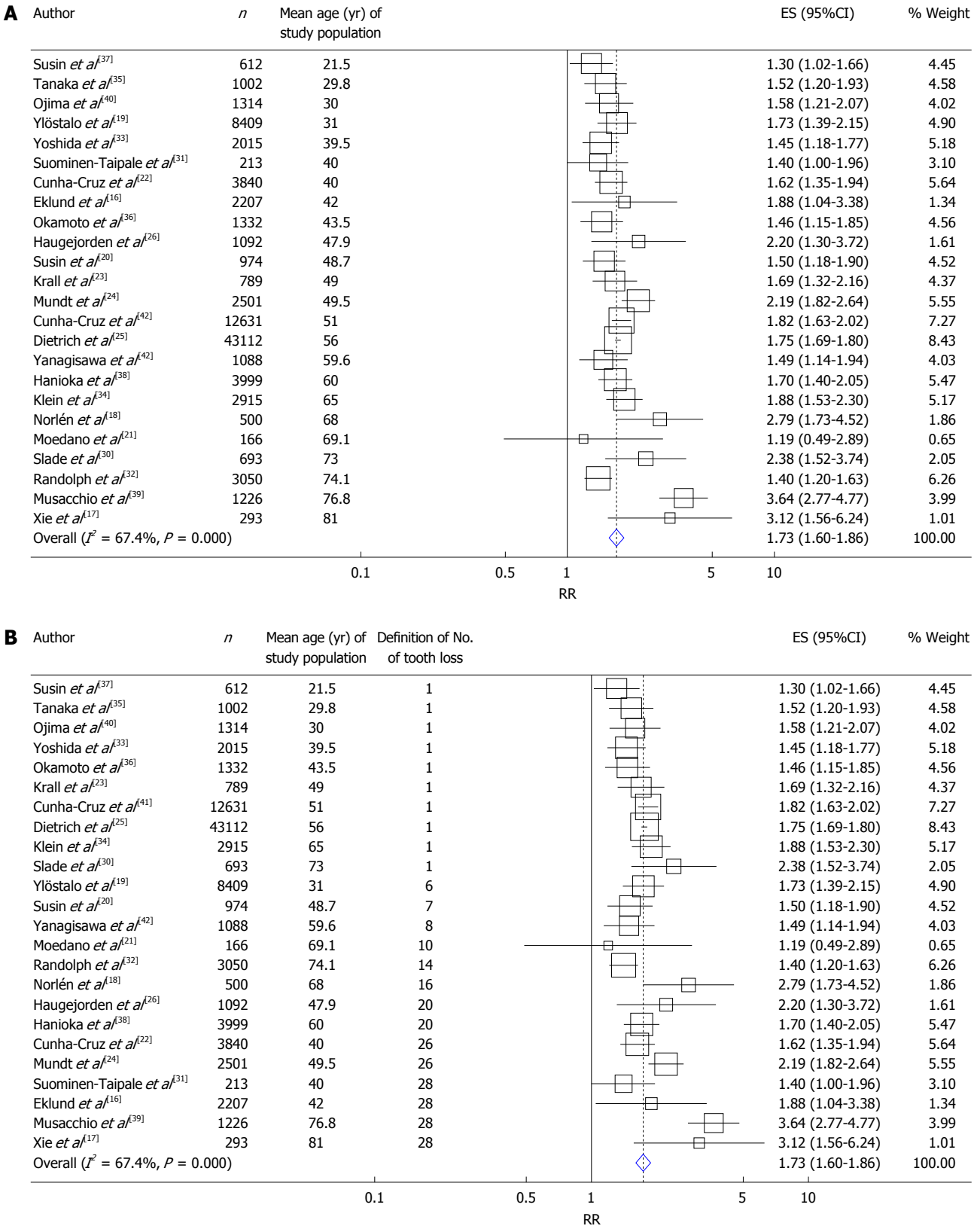

Figure 3 Forest plots of relative risk sorted by mean age of the study population (A) and by the number of teeth lost defined as representing a case (B). A: The size of the squares corresponds to the weight of the study in the meta-analysis. Combined relative risk was calculated using the random-effects model; B: The studies with definition of 1 means those losing one or more teeth were defined as cases. The size of the squares corresponds to the weight of the study in the meta-analysis. Combined relative risk was calculated using the random-effects model. Weights are from random effects analysis.

First, because we did not include data on smoking dose and duration, we could not exclude confounding by these factors. Some studies have indicated that the effect of smoking is dose- and duration-dependent[19,24,25,36]. Par-

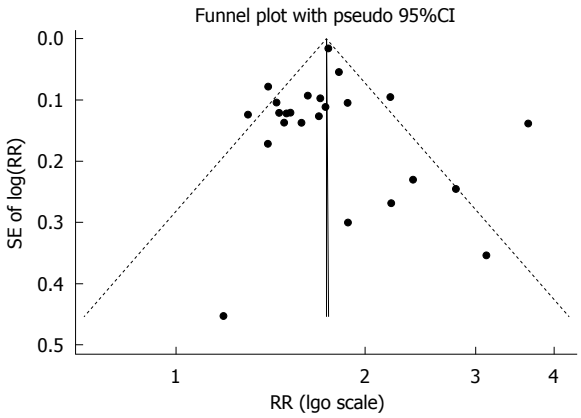

Figure 4 Funnel plot of included studies for the evaluation of publication bias.

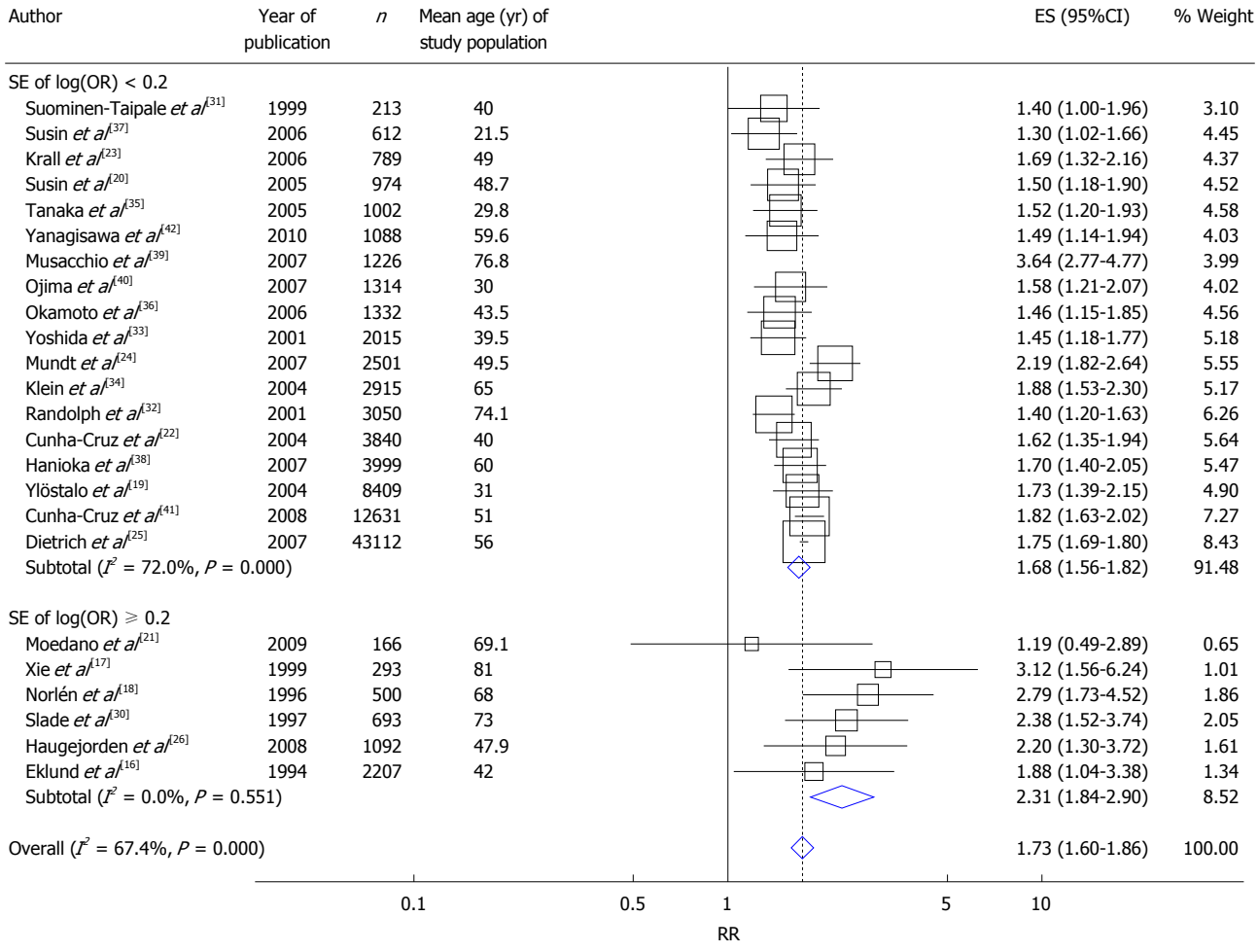

Figure 5 Subset-analysis according to the precision of studies. Weights are from random effects analysis.

ticularly among current smokers, older smokers may also have a lower daily consumption. Second, because tooth loss is a cumulative and irreversible event, older subjects may tend to have fewer teeth than younger smokers, and might therefore tend to be defined as case subjects. Third, chronic diseases such as diabetes and osteoporosis, which are considered as risk factors for tooth loss, may be more prevalent in older than younger people[17,21].

Several technical limitations of this meta-analysis warrant mention. One major limitation is the data source we used. Analyses were based on abstracted rather than individual patient data (IPD). In general, an IPD-based meta-analysis would allow a more robust estimation of the association. Second, the validity of meta-analyses is significantly threatened by potential publication bias. Although we detected no evidence of publication bias using

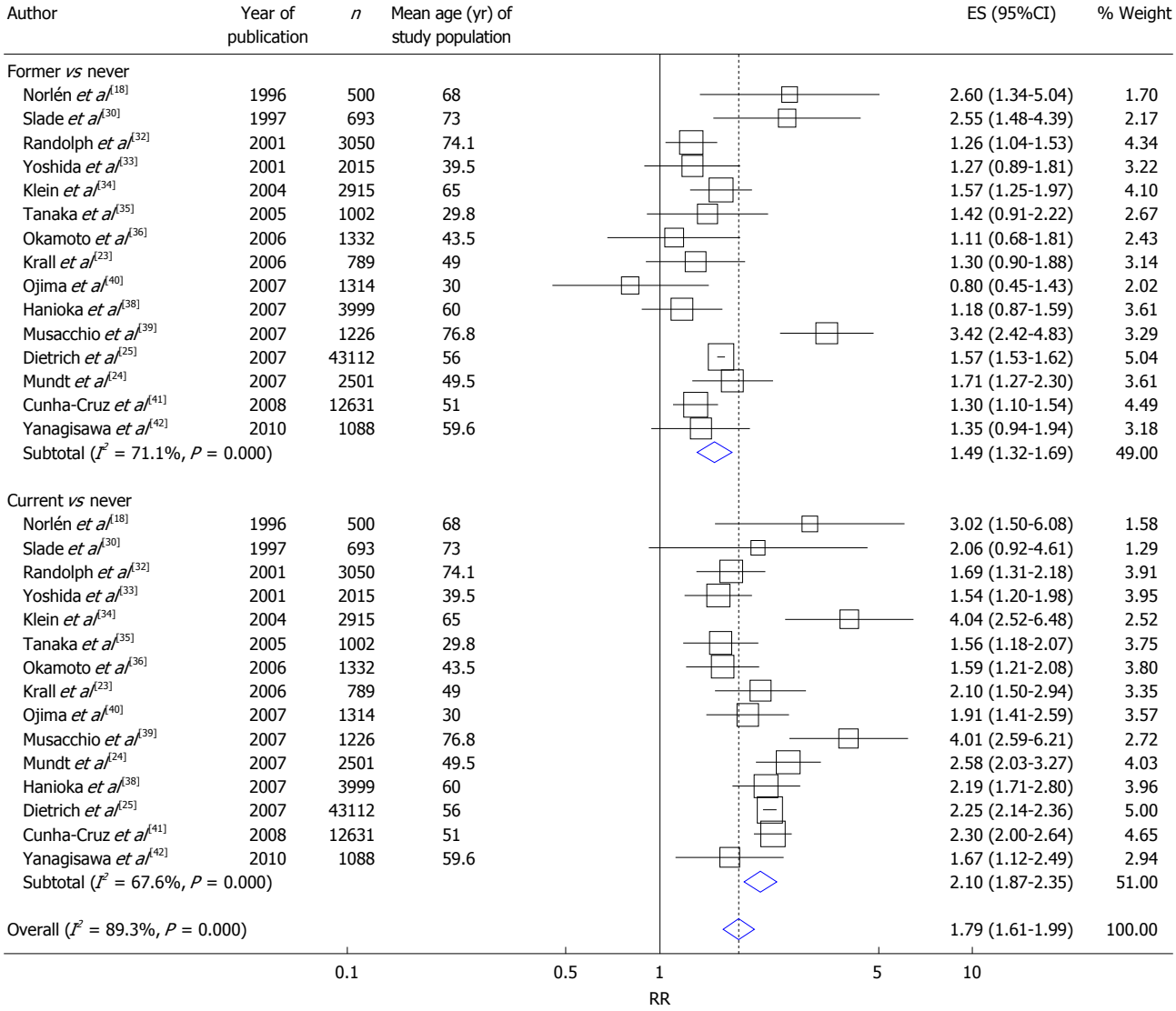

Figure 6 Subset meta-analysis according to smoking status. Weights are from random effects analysis.

graphical and statistical methods, it is difficult to completely rule out this possibility. A third limitation might be potential heterogeneity across studies, although we applied a random-effect model. Further, although we evaluated as many candidate characteristics as possible, unmeasured potential sources of heterogeneity might have remained. Although we tried to evaluate the different impact of smoking between former and current smokers, we did not directly compare two groups because RRs directly comparing two groups were not available in most of the studies. There have been discussions on how to precisely estimate the pooled estimates of RRs combining several levels of groups with strong correlations[48-50], we chose Greenland and Longnecker's methods[48] instead of Hamling's method[49] based on a recent study by Orsini *et al*[50] reporting negligible difference in estimation. Finally, we abstracted data only from English-language articles, and we only used PubMed search results because of lack of access. Therefore, bias might have occurred in our search strategy. However, given the nature of the studies we were looking for, namely clinical studies of adequate quality, we consider our search within MEDLINE to be sufficient.

In conclusion, we demonstrated that smoking is a risk factor for tooth loss regardless of many other confounders, and that smoking cessation has a protective effect against tooth loss. Although our conclusions should be interpreted cautiously, our results nevertheless raise a critical point regarding the long-standing debate on whether smoking is a risk factor for tooth loss. Implementation of smoking cessation in the dental field is encouraged.

COMMENTS

Background

The World Health Organization Global Oral Health Program works to increase awareness of oral health worldwide as an important component of general health and quality of life. Number of tooth loss is one of the main impediments to oral health and smoking behavior could be the one of the modifiable causes of tooth loss, therefore, quantitative evaluation of the impact of smoking on tooth loss is needed.

Research frontiers

Smoking behavior is a risk factor for the risk of tooth loss, however, the effects of confounders such as sex, age, and other comorbidity on tooth loss have

limited its interpretability among population. Therefore, more quantitative evaluation of the association between smoking and tooth loss is essential.

Innovations and breakthroughs

Previous studies have suggested that smoking behavior could be a risk of tooth loss, however, it has not been quantitatively evaluated. This meta-analysis of the literatures clarified that (1) ever-smoking increased the risk by 73% relative to non-smokers; and (2) risk increase among former smokers was different from that in current smokers (49% and 110%, compared to non-smokers). The latter suggests that it is important to consider smoking cessation to reduce the risk of tooth loss among smokers.

Applications

The study results suggest that the smoking increased the risk of tooth loss. Smoking cessation might be recommended to reduce the risk of tooth loss.

Peer review

This is a good quantitative study in which authors analyzed the impact of smoking on number of teeth loose with consideration of potential heterogeneity of studies. The results are interesting and suggest that smoking behavior should be considered in the oral health policy and practice.

REFERENCES

1 **Petersen PE**. World Health Organization global policy for improvement of oral health--World Health Assembly 2007. *Int Dent J* 2008; **58**: 115-121 [PMID: 18630105]

2 **You Z**, Cushman M, Jenny NS, Howard G. Tooth loss, systemic inflammation, and prevalent stroke among participants in the reasons for geographic and racial difference in stroke (REGARDS) study. *Atherosclerosis* 2009; **203**: 615-619 [PMID: 18801482 DOI: 10.1016/j.atherosclerosis.2008.07.037]

3 **Choe H**, Kim YH, Park JW, Kim SY, Lee SY, Jee SH. Tooth loss, hypertension and risk for stroke in a Korean population. *Atherosclerosis* 2009; **203**: 550-556 [PMID: 19013571 DOI: 10.1016/j.atherosclerosis.2008.07.017]

4 **Tu YK**, Galobardes B, Smith GD, McCarron P, Jeffreys M, Gilthorpe MS. Associations between tooth loss and mortality patterns in the Glasgow Alumni Cohort. *Heart* 2007; **93**: 1098-1103 [PMID: 17164486 DOI: 10.1136/hrt.2006.097410]

5 **Taguchi A**, Sanada M, Suei Y, Ohtsuka M, Lee K, Tanimoto K, Tsuda M, Ohama K, Yoshizumi M, Higashi Y. Tooth loss is associated with an increased risk of hypertension in postmenopausal women. *Hypertension* 2004; **43**: 1297-1300 [PMID: 15117916 DOI: 10.1161/01.HYP.0000128335.45571.ce]

6 **Desvarieux M**, Schwahn C, Völzke H, Demmer RT, Lüdemann J, Kessler C, Jacobs DR, John U, Kocher T. Gender differences in the relationship between periodontal disease, tooth loss, and atherosclerosis. *Stroke* 2004; **35**: 2029-2035 [PMID: 15256677 DOI: 10.1161/01.STR.0000136767.71518.36]

7 **Joshipura KJ**, Hung HC, Rimm EB, Willett WC, Ascherio A. Periodontal disease, tooth loss, and incidence of ischemic stroke. *Stroke* 2003; **34**: 47-52 [PMID: 12511749]

8 **Desvarieux M**, Demmer RT, Rundek T, Boden-Albala B, Jacobs DR, Papapanou PN, Sacco RL. Relationship between periodontal disease, tooth loss, and carotid artery plaque: the Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Stroke* 2003; **34**: 2120-2125 [PMID: 12893951 DOI: 10.1161/01.STR.0000085086.50957.22]

9 **Meyer MS**, Joshipura K, Giovannucci E, Michaud DS. A review of the relationship between tooth loss, periodontal disease, and cancer. *Cancer Causes Control* 2008; **19**: 895-907 [PMID: 18478344 DOI: 10.1007/s10552-008-9163-4]

10 **Hanioka T**, Ojima M, Tanaka K, Matsuo K, Sato F, Tanaka H. Causal assessment of smoking and tooth loss: a systematic review of observational studies. *BMC Public Health* 2011; **11**: 221 [PMID: 21477320 DOI: 10.1186/1471-2458-11-221]

11 **Joshipura KJ**, Willett WC, Douglass CW. The impact of edentulousness on food and nutrient intake. *J Am Dent Assoc* 1996; **127**: 459-467 [PMID: 8655866]

12 **Sheiham A**, Steele JG, Marcenes W, Tsakos G, Finch S, Walls AW. Prevalence of impacts of dental and oral disorders and their effects on eating among older people; a national survey in Great Britain. *Community Dent Oral Epidemiol* 2001; **29**: 195-203 [PMID: 11409678]

13 **Slade GD**, Spencer AJ. Social impact of oral conditions among older adults. *Aust Dent J* 1994; **39**: 358-364 [PMID: 7832683]

14 **Fiske J**, Davis DM, Frances C, Gelbier S. The emotional effects of tooth loss in edentulous people. *Br Dent J* 1998; **184**: 90-93; discussion 79 [PMID: 9489217]

15 **Gerritsen AE**, Allen PF, Witter DJ, Bronkhorst EM, Creugers NH. Tooth loss and oral health-related quality of life: a systematic review and meta-analysis. *Health Qual Life Outcomes* 2010; **8**: 126 [PMID: 21050499 DOI: 10.1186/1477-7525-8-126]

16 **Eklund SA**, Burt BA. Risk factors for total tooth loss in the United States; longitudinal analysis of national data. *J Public Health Dent* 1994; **54**: 5-14 [PMID: 8164192]

17 **Xie Q**, Ainamo A. Association of edentulousness with systemic factors in elderly people living at home. *Community Dent Oral Epidemiol* 1999; **27**: 202-209 [PMID: 10385358]

18 **Norlén P**, Johansson I, Birkhed D. Impact of medical and lifestyle factors on number of teeth in 68-year-old men in southern Sweden. *Acta Odontol Scand* 1996; **54**: 66-74 [PMID: 8669244]

19 **Ylöstalo P**, Sakki T, Laitinen J, Järvelin MR, Knuuttila M. The relation of tobacco smoking to tooth loss among young adults. *Eur J Oral Sci* 2004; **112**: 121-126 [PMID: 15056108 DOI: 10.1111/j.0909-8836.2004.00111.x]

20 **Susin C**, Oppermann RV, Haugejorden O, Albandar JM. Tooth loss and associated risk indicators in an adult urban population from south Brazil. *Acta Odontol Scand* 2005; **63**: 85-93 [PMID: 16134547]

21 **Moedano DE**, Irigoyen ME, Borges-Yáñez A, Flores-Sánchez I, Rotter RC. Osteoporosis, the risk of vertebral fracture, and periodontal disease in an elderly group in Mexico City. *Gerodontology* 2011; **28**: 19-27 [PMID: 19863666 DOI: 10.1111/j.1741-2358.2009.00342.x]

22 **Cunha-Cruz J**, Nadanovsky P, Faerstein E, Lopes CS. Routine dental visits are associated with tooth retention in Brazilian adults: the Pró-Saúde study. *J Public Health Dent* 2004; **64**: 216-222 [PMID: 15562944]

23 **Krall EA**, Dietrich T, Nunn ME, Garcia RI. Risk of tooth loss after cigarette smoking cessation. *Prev Chronic Dis* 2006; **3**: A115 [PMID: 16978490]

24 **Mundt T**, Schwahn C, Mack F, Polzer I, Samietz S, Kocher T, Biffar R. Risk indicators for missing teeth in working-age Pomeranians--an evaluation of high-risk populations. *J Public Health Dent* 2007; **67**: 243-249 [PMID: 18087995]

25 **Dietrich T**, Maserejian NN, Joshipura KJ, Krall EA, Garcia RI. Tobacco use and incidence of tooth loss among US male health professionals. *J Dent Res* 2007; **86**: 373-377 [PMID: 17384035]

26 **Haugejorden O**, Klock KS, Astrøm AN, Skaret E, Trovik TA. Socio-economic inequality in the self-reported number of natural teeth among Norwegian adults--an analytical study. *Community Dent Oral Epidemiol* 2008; **36**: 269-278 [PMID: 18474059]

27 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188 [PMID: 3802833]

28 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]

29 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563]

30 **Slade GD**, Gansky SA, Spencer AJ. Two-year incidence of tooth loss among South Australians aged 60+ years. *Community Dent Oral Epidemiol* 1997; **25**: 429-437 [PMID: 9429816]

31 **Suominen-Taipale AL**, Alanen P, Helenius H, Nordblad A, Uutela A. Edentulism among Finnish adults of working age, 1978-1997. *Community Dent Oral Epidemiol* 1999; **27**: 353-365 [PMID: 10503796]

32 **Randolph WM**, Ostir GV, Markides KS. Prevalence of tooth loss and dental service use in older Mexican Americans. *J*

Am Geriatr Soc 2001; **49**: 585-589 [PMID: 11380751]
33 **Yoshida Y**, Hatanaka Y, Imaki M, Ogawa Y, Miyatani S, Tanada S. Epidemiological study on improving the QOL and oral conditions of the aged--Part 2: Relationship between tooth loss and lifestyle factors for adults men. *J Physiol Anthropol Appl Human Sci* 2001; **20**: 369-373 [PMID: 11840690]
34 **Klein BE**, Klein R, Knudtson MD. Life-style correlates of tooth loss in an adult Midwestern population. *J Public Health Dent* 2004; **64**: 145-150 [PMID: 15341137]
35 **Tanaka K**, Miyake Y, Sasaki S, Ohya Y, Miyamoto S, Matsunaga I, Yoshida T, Hirota Y, Oda H. Active and passive smoking and tooth loss in Japanese women: baseline data from the osaka maternal and child health study. *Ann Epidemiol* 2005; **15**: 358-364 [PMID: 15840549 DOI: 10.1016/j.annepidem.2004.12.005]
36 **Okamoto Y**, Tsuboi S, Suzuki S, Nakagaki H, Ogura Y, Maeda K, Tokudome S. Effects of smoking and drinking habits on the incidence of periodontal disease and tooth loss among Japanese males: a 4-yr longitudinal study. *J Periodontal Res* 2006; **41**: 560-566 [PMID: 17076782 DOI: 10.1111/j.1600-0765.2006.00907.x]
37 **Susin C**, Haas AN, Opermann RV, Albandar JM. Tooth loss in a young population from south Brazil. *J Public Health Dent* 2006; **66**: 110-115 [PMID: 16711630]
38 **Hanioka T**, Ojima M, Tanaka K, Aoyama H. Relationship between smoking status and tooth loss: findings from national databases in Japan. *J Epidemiol* 2007; **17**: 125-132 [PMID: 17641448]
39 **Musacchio E**, Perissinotto E, Binotto P, Sartori L, Silva-Netto F, Zambon S, Manzato E, Corti MC, Baggio G, Crepaldi G. Tooth loss in the elderly and its association with nutritional status, socio-economic and lifestyle factors. *Acta Odontol Scand* 2007; **65**: 78-86 [PMID: 17453425 DOI: 10.1080/00016350601058069]
40 **Ojima M**, Hanioka T, Tanaka K, Aoyama H. Cigarette smoking and tooth loss experience among young adults: a national record linkage study. *BMC Public Health* 2007; **7**: 313 [PMID: 17976246 DOI: 10.1186/1471-2458-7-313]
41 **Cunha-Cruz J**, Hujoel PP, Maupome G, Saver B. Systemic antibiotics and tooth loss in periodontal disease. *J Dent Res* 2008; **87**: 871-876 [PMID: 18719216]
42 **Yanagisawa T**, Ueno M, Shinada K, Ohara S, Wright FA, Kawaguchi Y. Relationship of smoking and smoking cessation with oral health status in Japanese men. *J Periodontal Res* 2010; **45**: 277-283 [PMID: 19744265 DOI: 10.1111/j.1600-0765.2009.01233.x]
43 The Health Consequences of Smoking: A Report of the Surgeon General. Atlanta, GA: Centers for Disease Control and Prevention (US), 2004
44 **Preshaw PM**, Heasman L, Stacey F, Steen N, McCracken GI, Heasman PA. The effect of quitting smoking on chronic periodontitis. *J Clin Periodontol* 2005; **32**: 869-879 [PMID: 15998271 DOI: 10.1111/j.1600-051X.2005.00779.x]
45 **Delima SL**, McBride RK, Preshaw PM, Heasman PA, Kumar PS. Response of subgingival bacteria to smoking cessation. *J Clin Microbiol* 2010; **48**: 2344-2349 [PMID: 20410352 DOI: 10.1128/JCM.01821-09]
46 **Shchipkova AY**, Nagaraja HN, Kumar PS. Subgingival microbial profiles of smokers with periodontitis. *J Dent Res* 2010; **89**: 1247-1253 [PMID: 20739702 DOI: 10.1177/0022034510377203]
47 **Niederman R**. Causal association between smoking and tooth loss is highly likely. *Evid Based Dent* 2011; **12**: 77 [PMID: 21979768 DOI: 10.1038/sj.ebd.6400809]
48 **Greenland S**, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* 1992; **135**: 1301-1309 [PMID: 1626547]
49 **Hamling J**, Lee P, Weitkunat R, Ambühl M. Facilitating meta-analyses by deriving relative effect and precision estimates for alternative comparisons from a set of estimates presented by exposure level or disease category. *Stat Med* 2008; **27**: 954-970 [PMID: 17676579 DOI: 10.1002/sim.3013]
50 **Orsini N**, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. *Am J Epidemiol* 2012; **175**: 66-73 [PMID: 22135359 DOI: 10.1093/aje/kwr265]

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Effectiveness of rehabilitation based on recreational activities: A systematic review

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Abstract

AIM: To summarize the evidence from randomized controlled trials (RCTs) on the rehabilitation effects of recreational activities.

METHODS: Studies were eligible if they were RCTs. Studies included one treatment group in which recreational activity was applied. We searched the following databases from 1990 to May 31, 2012: MEDLINE *via* PubMed, CINAHL, Web of Science, and Ichushi-Web. We also searched all Cochrane Databases and Campbell Systematic Reviews up to May 31, 2012.

RESULTS: Eleven RCTs were identified, which included many kinds of target diseases and/or symptoms such as stroke, dementia, Parkinson's disease, acquired brain injury, chronic non-malignant pain, adolescent obesity, high-risk pregnancy, and the frail elderly. Various intervention methods included gaming technology, music, dance, easy rider wheelchair biking, leisure education programs, and leisure tasks. The RCTs conducted have been of relatively low quality. A meta-analysis (pooled sample; n = 44, two RCTs) for balance ability using tests such as "Berg Balance Scale" and "Timed Up and Go Test" based on game intervention revealed no significant difference between interventions and controls. In all other interventions, there were one or more effects on psychological status, balance or motor function, and adherence as primary or secondary outcomes.

CONCLUSION: There is a potential for recreational activities to improve rehabilitation-related outcomes, particularly in psychological status, balance or motor function, and adherence.

Key words: Recreation activities; Randomized controlled trial; Rehabilitation effect

Core tip: This is the first systematic review of the effectiveness of rehabilitation based on recreational ac-

tivities. There is a potential for recreational activities to improve rehabilitation-related outcomes, particularly in psychological status (depression, mood, emotion, and power), balance or motor function, and adherence (feasibility and attendance). To most effectively assess the potential benefits of recreational activities for rehabilitation, it will be important for further research to utilize (1) randomized controlled trials methodology (person unit or cluster unit) when appropriate; (2) an intervention dose; (3) a description of adverse effects and withdrawals; and (4) the cost of recreational activities.

Kamioka H, Tsutani K, Yamada M, Park H, Okuizumi H, Honda T, Okada S, Park SJ, Kitayuguchi J, Handa S, Mutoh Y. Effectiveness of rehabilitation based on recreational activities: A systematic review. *World J Meta-Anal* 2013; 1(1): 27-46 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i1/27.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i1.27

INTRODUCTION

Recreational activity is anything that is stimulating and rejuvenating for an individual. Some people may enjoy nature hikes, others may enjoy playing the guitar. The idea behind these activities is to expand the mind and body in a positive, healthy way. The best reason to take part in these activities is that they will slow the aging process by helping to lessen or eliminate stress[1]. A dictionary describes "recreation" as "the fact of people doing things for enjoyment, when they are not working"[2]. However, there are various views about what constitutes a recreational activity and there is no fixed consensus.

A systematic review (SR) of randomized controlled trials (RCTs) based on recreational and leisure activity reported some beneficial effects such as improvement in quality of life, as well as health-promoting, educational and therapeutic effects[3]. Three RCTs adopted for this review evaluated appreciation of performed music[4], entertainment using an easy rider wheelchair bike[5], and leisure tasks[6] as the intervention method. In the present study, we assumed that recreational activity is defined broadly as a physical activity with a strong element of pleasure or enjoyment.

Stroke is a disease that typically requires rehabilitation and has been described as a worldwide epidemic[7]. Many stroke patients suffer from sensory, motor and cognitive impairment as well as a reduced ability to perform self care and to participate in social and community activities[8]. Standardized repetitive task training has been shown to be effective in some aspects of rehabilitation, such as improving walking distance and speed[9]. Over the years, virtual reality (VR) and interactive video gaming (IVG) have emerged as new treatment approaches in stroke rehabilitation. In particular, commercial gaming consoles are being rapidly adopted in clinical and nursing settings. A recent SR of stroke rehabilitation studies reported that the use of VR and IVG may be beneficial in improving arm function and activities of daily living (ADL) function when compared with the same dose of conventional therapy, although there was insufficient evidence to reach a conclusion about the effect of VR and IVG on grip strength or gait speed[10].

The current study has shown that even a short duration of Wii play can provide an effective adjunct to standard rehabilitation for fall prevention, although a "Wii only" training approach is not being advocated[11]. The "enjoyment" factor is an important one that may aid adherence to training for rehabilitation[12].

Low back pain is also a disease that requires rehabilitation and is the most common reason for use of complementary and alternative medicine in the United States[13]. A SR of RCTs into alternative therapy (*i.e.,* spa and balneotherapy) targeting the relief of lower back pain reported that even though the data are scarce, there was encouraging evidence suggesting that these therapies may be effective[14].

Over the years, recreational activity and relaxation in a forest environment called "forest therapy" or "Shinrin-yoku" (forest-air bathing and forest-landscape watching, walking, *etc.*), have become a kind of climate therapy or nature therapy and are popular methods for many urban people with mentally stressful situations[15]. The fields of preventive and alternative medicine have also shown an interest in the therapeutic effects of forest therapy[16]. A study reported that forest environments may contribute to the maintenance of health and well-being by, for example reducing hostility and depression which are risk factors for coronary heart diseases, or by improving overall emotions, particularly among populations with poor mental health[17]. In addition, a recent study reported that forest bathing trips increase natural killer (NK) cell activity, which was mediated by increases in the number of NK cells and by the levels of intracellular anti-cancer proteins and phytoncides released from trees. The decreased production of stress hormones may also partially contribute to the increased NK cell activity[18].

It is well known in research design that evidence grading is highest for a SR with meta-analysis of RCTs. Although many studies have reported the rehabilitation effects of recreational activities, there is no SR of evidence based on RCTs. The objective of this review was to summarize the evidence from RCTs on the rehabilitation effects of recreational activities.

MATERIALS AND METHODS

Criteria for considering studies included in this review

Types of studies: Studies were eligible if they were RCTs.

Types of participants: There was no restriction on patients.

Types of intervention and language: Studies included

at least one treatment group in which recreation activity was applied. The definition of the recreational activity is complex, but, in this study, it describes a specific exercise item. Specifically, any kind of recreational activity (not only dynamic activities but also musical appreciation or playing, painting, hand-craft, *etc.*) was permitted and defined as an intervention. However, we excluded comprehensive exercise interventions such as walking, jogging, Tai chi, Yoga, stretching, and strength training. There was no restriction on the basis of language.

Types of outcome measures: We focused on rehabilitation effects. The World Health Organization states that rehabilitation of people with disabilities is a process aimed at enabling them to reach and maintain their optimal physical, sensory, intellectual, psychological and social functional levels[19]. Rehabilitation provides disabled people with the tools they need to attain independence and self-determination. In this study, beneficial outcome measures included cognitive function, physical function, and pain-relief. We did not specify secondary outcomes but instead estimated items as primary outcomes if an article treated them as rehabilitation effects.

Search methods for studies identification

Bibliographic database: We searched the following databases from 1990 to May 31, 2012: MEDLINE *via* PubMed, CINAHL, Web of Science, Ichushi Web (in Japanese), and the Western Pacific Region Index Medicus (WPRIM). The International Committee of Medical Journal Editors (ICMJE) recommended uniform requirements for manuscripts submitted to biomedical journals in 1993. We selected articles published (that included a protocol) since 1990, because it appeared that the ICMJE recommendation had been adopted by the relevant researchers and had strengthened the quality of reports.

We also searched the Cochrane Database of Systematic Reviews (Cochrane Reviews), the Database of Abstracts of Reviews of Effects (Other Reviews), the Cochrane Central Register of Controlled Trials (Clinical Trials or CENTRAL), the Cochrane Methodology Register (Methods Studies), the Health Technology Assessment Database (Technology Assessments), the NHS Economic Evaluation Database (Economic Evaluations), About The Cochrane Collaboration databases (Cochrane Groups) and Campbell Systematic Reviews (the Campbell Collaboration), and the All Cochrane all up to May 31, 2012.

All searches were performed by two specific searchers (hospital librarians) who were qualified in medical information handling, and who had sophisticated skills in the searching of clinical trials.

Search strategies: The special search strategies contained the elements and terms for MEDLINE, CINAHL, Web of Science, Ichushi Web, WPRIM, All Cochrane databases, and Campbell Collaboration (Table 1). Only keywords about interventions were used for the searches. First, titles and abstracts of identified published articles were reviewed in order to determine the relevance of the articles. Next, references in relevant studies and identified RCTs were screened.

Registry checking: We searched the International Clinical Trials Registry Platform (ICTRP), Clinical Trials.gov, the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR), the Japan Pharmaceutical Information Center-Clinical Trials Information (Japic CTI), and the Japan Medical Association-Center for Clinical Trials (JMACCT CTR), all up to May 31, 2012. ICTRP and the WHO Registry Network meet specific criteria for content, quality and validity, accessibility, unique identification, technical capacity and administration. Primary registries meet the requirements of the ICMJE. Clinical Trials.gov is a registry of federally and privately supported clinical trials conducted in the United States and around the world. UMIN-CTR, Japic CTI, and JMACCT CTR are registries of clinical trials conducted in Japan and around the world.

Handsearching, reference checking, *etc.*: We handsearched abstracts published on recreation activities in relevant journals in Japan. We checked the references of included studies for further relevant literature.

Review methods

Selection of trials: In order to make the final selection of studies for the review, all criteria were applied independently by five authors (*e.g.,* Honda T, Kitayuguchi J, Okada S, Park SJ) to the full text of articles that had passed the first eligibility screening (Figure 1). Disagreements and uncertainties were resolved by discussion with other authors (*e.g.*, Mutoh Y, Okuizumi H, Park H).

Studies were selected when (1) the design was an RCT; and (2) one of the interventions was a form of recreational activity. Rehabilitation effects were used as a primary outcome measure. Trials that were excluded are presented with reasons for their exclusion (Table 2).

Quality assessment of included studies

In order to ensure that variation was not caused by systematic errors in the study design or execution, seven review authors (Okuizumi H, Mutoh Y, Okada S, Park SJ, Honda T, Handa S, and Honda T) independently assessed the quality of articles. A full quality appraisal of these papers was made using the combined tool based on the "CONSORT 2010"[20] and the "CONSORT for non-pharmacological trials"[21], developed to assess the methodological quality of non-pharmacological RCTs. These checklists were not originally developed to use as a quality assessment instrument, but we used them because they are the most important tools related to the internal and external validity of trials.

Each item was scored as "present" (p), "absent" (a), "unclear or inadequately described" (?), or "not appli-

Table 1 The special search strategies

1 MEDLINE
#1 Search "Recreation" [MeSH Major Topic]
#2 Search "Recreation Therapy" [MeSH Major Topic]
#3 Search "Rehabilitation" [MeSH Major Topic]
#4 Search "Treatment Outcome" [MeSH Terms]
#5 Search (#1) OR #2
#6 Search (#3) OR #4
#7 Search (#5) AND #6
#8 Search (#5) AND #6 Filters: Publication date from 1990-01-01 to 2012-04-30
#9 Search (#5) AND #6 Filters: Publication date from 1990-01-01 to 2012-04-30; Humans
#10 Search (#5) AND #6 Filters: Publication date from 1990-01-01 to 2012-04-30; Humans; Randomized Controlled Trial
2 CINHAL
#1 TX recreation
#2 (MH "Recreation+") OR (MH "Recreational Therapy")
#3 #1 or #2
#4 TX rehabilitation
#5 (MH "Rehabilitation+")
#6 (MH "Treatment Outcomes+") OR (MH "Outcome Assessment")
#7 #4 or #5 or #6
#8 #3 and #7
#9 #3 and #7
#10 #3 and #7
#11 #3 and #7
3 Web of Science
#1 Recreation
#2 Leisure
#3 #1 OR #2
#4 Rehabilitation
#5 "Quality of life"
#6 Outcome
#7 #4 OR #5 OR #6
#8 #3 AND #7
#9 Randomized OR randomised
#10 (#8 AND #9) AND Article time span = 1990-2012
4 Ichushi Web (Originally in Japanese)
#1 Recreation/TH or recreation/AL or recreational/AL or recreation/AL or Rikuryeshon/AL or recreation/AL
#2 Rehabilitation/HL or rehabilitation/AL or rehabilitation/ALAL
#3 #1 and #2
#4 (#3) and (DT = 1990:2012 PT = original papers CK = person)
#5 (#4) and (RD = randomized controlled trials, quasi-randomized controlled trials, comparative studies)
#6 (#4) and (RD = randomized controlled trials)
5 WPRIM
#1 recreation
6 All Cochrane
#1 MeSH descriptor Recreation explode all trees
#2 (recreation): ti, ab, kw
#3 MeSH descriptor Rehabilitation explode all trees
#4 MeSH descriptor Randomized Controlled Trials as Topic explode all trees
#5 (Randomized controlled trial): ti, ab, kw
#6 (#1 OR #2)
#7 (#4 OR #5)
#8 (#3 AND #6 AND #7), from 1990 to 2012
7 Campbell Collaboration
#1 Recreation
8 ICTRP
#1 Recreation
9 Clinical Trials.gov
#1 Recreation OR recreational
10 UMIN-CTR (Originally in Japanese)
#1 Recreation
11 Japic CTI (Originally in Japanese)
#1 Recreation
12 JMACCT CTR (Originally in Japanese)
#1 Recreation

ICTRP: International Clinical Trials Registry Platform; UMIN-CTR: University Hospital Medical Information Network-Clinical Trials Registry; Japic CTI: Japan Pharmaceutical Information Center-Clinical Trials Information; JMACCT CTR: Japan Medical Association-Center for Clinical Trials.

cable" (n/a). Depending on the study design, some items were not applicable. The "n/a" studies were excluded from calculation for quality assessment. We displayed the percentage of present description in all 47 checked items for the quality assessment of articles. Then, based on the percentage of risk of poor methodology and/or bias, each item was assigned to the following categories: good description (80%-100%), poor description (50%-79%), very poor description (0%-49%). Disagreements and uncertainties were resolved by discussion with other authors (*e.g.,* Okuizumi H, Okada S and Kamioka H). Inter-rater reliability was calculated on a dichotomous scale using percentage agreement and Cohen's κ coefficient (*k*).

Summary of studies and data extraction: Seven review authors (Okuizumi H, Mutoh Y, Okada S, Park SJ, Honda T, Handa S and Kamioka H) described the summary from each article based on the recommended structured abstracts[22,23].

Benefit, harm, and withdrawals

The GRADE Working Group[24] reported that the balance between benefit and harm, quality of evidence, applicability, and the certainty of the baseline risk were all considered in judgments about the strength of recommendations. Adverse events, withdrawals, and cost for intervention were especially important information for researchers and users of clinical practice guidelines, and we have presented this information with the description of each article.

Analysis

Pre-planned stratified analyses were: (1) trials comparing recreational activities with no treatment or waiting list controls; (2) trials comparing different types of general rehabilitation method [*e.g.,* physical therapy, occupational therapy (OT), *etc.*]; and (3) trials comparing recreational activities with other intervention(s) (*e.g.,* musical appreciation *vs* singing). We planned to express the results of each RCT, when possible, as relative risk with corresponding 95%CI for dichotomous data, and as standardized or weighted mean differences (SMD) with 95%CI for continuous data. However, heterogeneous results of studies that met inclusion criteria were not combined. All analyses were computed with the "R version 2.15.1", a free software environment for statistical computing and graphics (URL:http://www.r-project.org/), which compiles and runs on a wide variety of UNIX platforms, Windows.

Table 2 References to studies excluded in this review

Excusion No.	Ref.	Title	Reason of exclusion
1	Green *et al*[48]	Physiotherapy for patients with mobility problems more than 1 year after stroke: a randomised controlled trial	Not recreation activity
2	Kobayashi *et al*[49]	Effects of a fall prevention program on physiacal activities of elderly people living in a rural region: an interventional trial	Community-dwelling healthy elderly
3	Das *et al*[50]	The efficacy of playing a virtual reality game in modulating pain for children with acute burn injuries: A randomized controlled trial	Cross-over design
4	Hurwitz *et al*[51]	Effects of recreational physical activity and back exercises on low back pain and psychological distress: Findings from the UCLA low back pain study	Observational study
5	Matsuo[52]	The influence of the exercise using a video game on the physical function and brain activities	Cross-over design
6	Saposnik *et al*[53]	Effectiveness of virtual reality exercises in stroke rehabilitation: rationale, design, and protocol of a pilot randomized clinical trial assessing the Wii gaming system	Research protocol
7	Mitsumura *et al*[54]	Effect on physical and mental function of a group rhythm exercise for elderly persons certified under the less severe grades of long-term care insurance	Exercise training
8	Fraga *et al*[55]	Aerobic resistance, functional autonomy and quality of life of elderly women impacted by a recreation and walking program	Community-dwelling healthy elderly
9	Watanabe *et al*[56]	Effects of congnitive rehabilitation with computer training on neurophychological function in schizophrenia	Not recreation activity
10	Hsu *et al*[57]	A "Wii" bit of fun: The effects of adding Nintendo Wii Bowling to a standard exercise regimen for residents of long-term care with upper extremity dysfunction	Cross-over design
11	Kwok *et al*[58]	Evaluation of the Frails' Fall Efficacy by Comparing Treatments on reducing fall and fear of fall in moderately frail older adults: study protocol for a randomised control trial	Research protocol

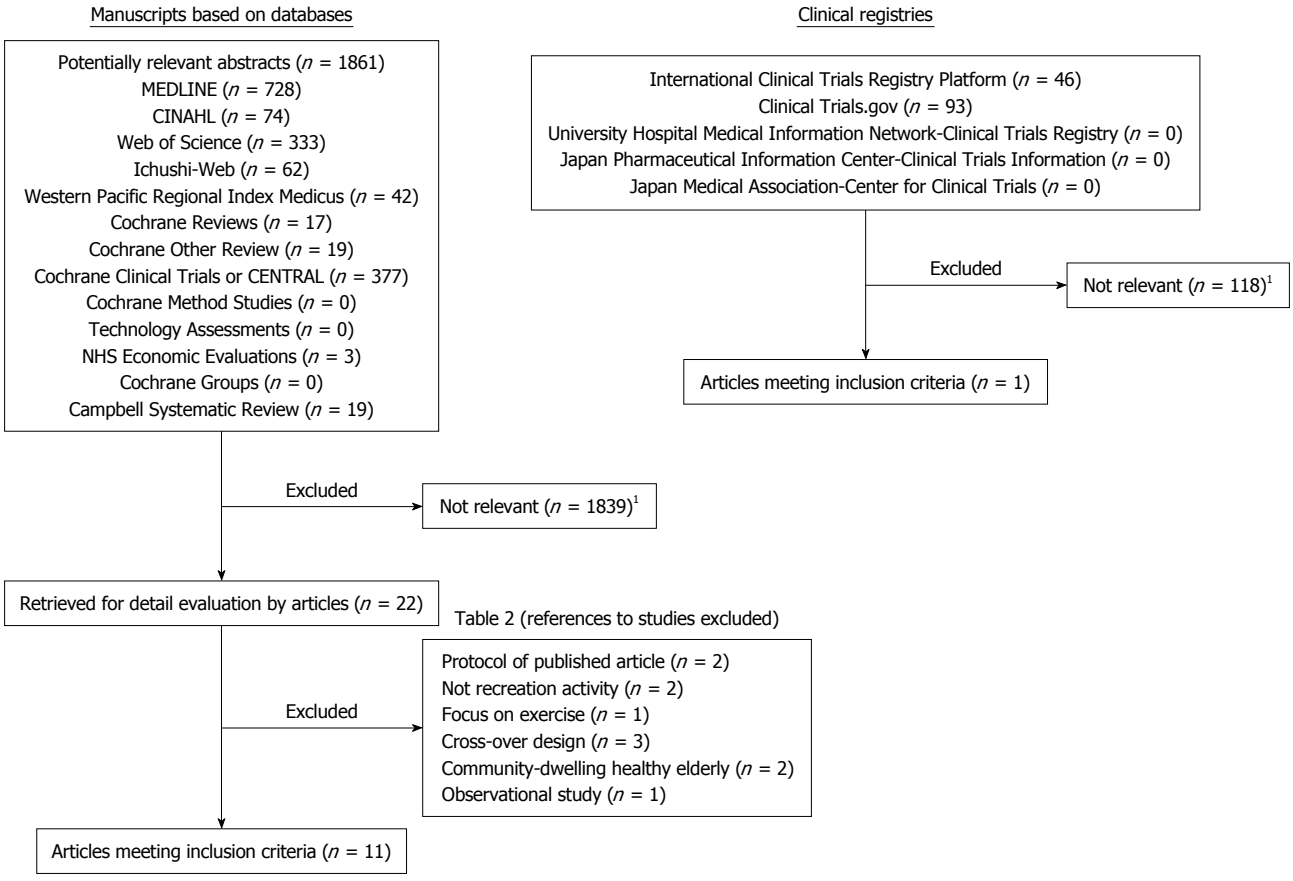

Figure 1 Flowchart of trial process. [1]Reduplication.

Research protocol registration

We submitted and registered our research protocol to the PROSPERO database (No. CRD42012002381)[25]. This is an international database of prospectively registered SRs in health and social care. Key features from the review protocol are recorded and maintained as a permanent record in PROSPERO. This will provide a comprehensive listing of SRs registered at inception, and enable compar-

ison of reported review findings with what was planned in the protocol. PROSPERO is managed by CRD and funded by the UK National Institute for Health Research. Registration was recommended because it encourages full publication of the review's findings and transparency in changes to methods that could bias findings[26].

RESULTS

Study selection

The literature searches based on databases included 1861 potentially relevant articles (Figure 1). Abstracts from those articles were assessed and 22 papers were retrieved for further evaluation (checks for relevant literature). Eleven publications were excluded because they did not meet the eligibility criteria (Table 2). Eleven studies[4-6,27-34] met all inclusion criteria (Figure 1).

Study characteristics

The language of all eligible publications was English. Target diseases and/or symptoms (Table 3) were stroke,[6,29,33,34] depression[5], Parkinson's disease[32], acquired brain injury[28], chronic non-malignant pain (CNMP)[4], adolescent obesity[31], high-risk pregnancy[30], and the frail elderly[27]. Intervention methods were gaming technology[27-29,31,33], music[4,30], dance[32], easy rider wheelchair biking[5], leisure education programs[34], and leisure tasks[6].

For gaming technology intervention, Szturm *et al*[27] reported that dynamic balance exercises on fixed and compliant sponge surfaces could be coupled to interactive video game-based tasks in frail community-dwelling older adults. Gil-Gómez *et al*[28] reported that virtual treatment with game exercises promotes improvement in the dynamic balance of patients with acquired brain injury. Saposnik *et al*[29] reported that VR Wii gaming technology represents a safe, feasible, and potentially effective alternative to facilitate rehabilitation therapy and promote motor recovery after stroke. Adamo *et al*[31] reported that cycling to music was superior to interactive video game cycling in promoting attendance and intensity of exercise expenditure for obese adolescent people, indicating that investment in the more expensive GameBike may not be worth the cost. Yavuzer *et al*[33] reported that Playstation EyeToy Games combined with a conventional stroke rehabilitation program have the potential to enhance upper extremity-related motor functioning in subacute stroke patients.

Siedliecki *et al*[4] reported that nurses could help patients with CNMP identify and use music they enjoy as a self-administered complementary intervention to facilitate feelings of power, and to decrease perceptions of pain, depression and disability. Bauer *et al*[30] reported that single session music and recreational therapy interventions effectively alleviate antepartum-related distress among high-risk women experiencing antepartum hospitalization and should be considered as valuable additions to any comprehensive antepartum program.

Concerning dance intervention, Hackney *et al*[32] reported that the tango may target deficits associated with Parkinson's disease more than the waltz/foxtrot, but both dances may benefit balance and locomotion.

Fitzsimmons[5] reported that easy rider wheelchair biking contributed to the body of knowledge regarding options for the treatment of depression in older adults, and provided encouraging findings that psychosocial interventions might be effective in reducing depression.

Desrosiers *et al*[34] reported that the results for leisure education programs indicated their effectiveness in improving participation in leisure activities, improving satisfaction with leisure and reducing depression in people with stroke.

Parker *et al*[6] reported that additional OT treatments did not show a clear beneficial effect on mood, leisure activity or independence in ADL measured at 6 or 12 mo.

Quality assessment

We evaluated 47 items from the CONSORT 2010 and the "CONSORT for non-pharmacological trials" checklists in more detail (Table 4). Inter-rater reliability metrics for the quality assessment indicated substantial agreement for all 517 items (percentage agreement 97% and k = 0.953).

This assessment evaluated the quality of how the main findings of the study were summarized in the written report. There was a remarkable lack of description in the studies of the methods, results, discussion, and other information in general. The items for which the description was lacking (very poor; < 50%) in many studies were as follows (present ratio; %): "in the abstract, description of the experimental treatment, comparator, care providers, centers, and blinding status" (36%); "important changes to methods after trial commencement" (36%); "details of how the interventions were standardized" (40%); "details of how adherence of care providers with the protocol was assessed or enhanced" (11%); "any changes to trial outcomes after the trial outcomes after the trial commenced" (25%); "how sample size was determined" (45%); "when applicable, explanation of any interim analyses and stopping guidelines" (22%); "when applicable, details of whether and how the clustering by care providers or centers was addressed" (11%); "type of randomization" (29%); "when applicable, how care providers were allocated to each trial group" (29%); "who generated the random allocation sequence, who enrolled participants, and who assigned participants to intervention" (45%); "whether or not those administering co-interventions were blinded to group assignment" (0%); "if blinded, method of blinding and description of the similarity of interventionist" (18%); "methods for additional analyses, such as subgroup analyses and adjusted analyses" (38%); "when applicable, details of whether and how the clustering by care providers or centers was addressed" (38%); "for binary outcomes, presentation of both absolute and relative effect sizes is recommended"

Table 3 Brief summary of articles based on structured abstracts and additional elements

Ref. Citation	Szturm *et al*[27] *Phys Ther* 2011; 91: 1449-1462	Gil-Gómez *et al*[28] *J Neuroeng Rehabil* 2011; 8: 30	Saposnik *et al*[29] *Stroke* 2010; 41: 1477-1484
Title	Effects of an interactive computer game exercise regimen on balance impairment in frail community-dwelling older adults: a randomized controlled trial	Effectiveness of a Wii balance board-based system (eBaViR) for balance rehabilitation: a pilot randomized clinical trial in patients with ABI	Effectiveness of virtual reality using Wii gaming technology in stroke rehabilitation: a pilot randomized clinical trial and proof of principle
Aim/objective	To examine the feasibility and benefits of physical therapy based on a task-oriented approach delivered *via* an engaging, interactive video game paradigm. The intervention focused on performing targeted dynamic tasks, which included reactive balance controls and environmental interaction	To evaluate the efficacy of the eBaViR system as a rehabilitation tool for balance recovery in patients with ABI	To examine the feasibility and safety the VR Nintendo Wii gaming system (VRWii) compared with RT in facilitating motor function on the upper extremity required for activities of daily living among patients with subacute stroke receiving standard rehablitation
Setting/place	A geriatric day hospital (Winnipeg, Manitoba, Canada)	Hospital NISA Valencia al Mar y Sevilla A ljarafe, Spain	Toronto Rehabilitation Institute
Participants	Thirty community-dwelling and ambulatory older adults. Inclusion Criteria; age: 65-85 yr, MMSE score > 24, English-speaking with the ability to understand the nature of the study and provide informed consent, independent in ambulatory functions, with or without an assistive device (cane or walker). without a disability and medical conditions (cancer, kidney disease, fracture, uncontrolled diabetes or seizure disorder, cardiovascular-related problems, stroke, multiple sclerosis, late-stage Parkinson disease, fainting, or dizzy spells)	Twenty participants. Inclusion criteria were: (1) age ⩾ 16 yr and < 80 yr; (2) chronicity > 6 mo; (3) absence of cognitive impairment (MMSE > 23); (4) able to follow instructions; and (5) ability to walk 10 m indoors with or without technical orthopaedic aids	Participants (n = 22) who are 18 to 85 yr of age (mean age 61.3 yr) having a first-time ischemic or hemorrhagic stroke
Intervention	The control group received the typical rehabilitation program such as strengthening and balance exercise at the day hospital. The experimental group received a program of dynamic balance exercises coupled with computer-based video game play, using a center-of-pressure position signal as the computer mouse. The tasks were performed while standing on a fixed floor surface, with progression to a compliant sponge pad. Each group received 16 sessions, scheduled 2/wk, with 45 min	Each patient participated in a total of 20 1-h-sessions of rehabilitation and accomplished a minimum of 3 sessions and a maximum of 5 sessions per week. During control sessions, traditional rehabilitation exercises that focused on balance training were practiced either individually or in a group. The sessions of the trial group were programmed according to the three games of the system (Simon, Balloon Breaker and Air Hockey) with a system based on the eBaViR. The eBaViR using Nintendo system had a significant improvement in static and/or standing balance (BBS and Anterior Reaches Test) compared to patients who underwent traditional therapy. The patients reported having had fun during the treatment without suffering from cyber side effects, which implies additional motivation and adhesion level to the treatment	Participants received an intensive program consisting of 8 interventional sessions of 60 min each over a 14-d period. Intervention group conducted a virtual reality Wii gaming, and the control group did a RT such as card game
Main and secondary outcomes	BBS, TUG, ABC	BBS, Brunel Balance Assessment, and ART	Feasibility and safety were set as the main outcome, and the efficiency was a secondary outcome in this study
Randomisation	Group assignment codes were placed in envelopes and sealed. Each individual who agreed to enter the study randomly selected an envelope	The randomization schedule was computer generated using a basic random number generator	The randomization schedule was computer generated using a basic random number generator
Blinding/masking	Assessors were blinded to the participant group assignments. The participant names of the GaitRite data files were coded	Program specialsits and assessors were blinded to the patients group assignments	Only caregivers were blinded (single blinding)
Numbers randomised	Experimental group (n = 15) and Control group (n = 15)	Trial group (n = 10) and Control group (n = 10)	Virtual Reality Therapy (n = 11) and Recreation Therapy (n = 11)

Recruitment	Thirty community-dwelling and ambulatory older adults who were attending the Riverview Health Center Day Hospital for treatment of limitations were recruited to participate in this study	"Seventy-nine hemiparetic patients who had sustained an ABI and were attending a rehabilitation program were potential candidates for participation in this study"	110 potential candidates were screened to participate in EVREST (the Effectiveness of Virtual Reality Excercises in Stroke Rehabiritation), and a total of 88 patients were excluded
Numbers analysed	Experimental group (n = 14) and Control group (n = 13)	Trial group (n = 9) and Control group (n = 8)	Virtual Reality Therapy (n = 10) and Recreation Therapy (n = 10) on the primary end point
Outcome	Finding demonstrated significant improvements in posttreatment balance performance scores for both group, and change scores were significantly greater in the experimental group compared with the control group (BBS; P = 0.001, ABC; P = 0.02). No significant treatment effect was observed in either group for the TUG or spatiotemporal gait variables	Patients using eBaViR had a significant improvement in static balance (P = 0.011 in BBS and P = 0.011 in ART) compared to patients who underwent traditional therapy. Regarding dynamic balance, the results showed significant improvement over time in all these measures, but no significant group effect or group-by-time interaction was detected for any of them, which suggest that both groups improved in the same way	Feasibility (time tolerance) and safety (intervention-related adverse event) did not show significant difference between groups. In contrast, the intervention group showed a significant improvement in mean motor function (Wolf Motor Function Test) compared to the control group (-7.4 s; 95%CI: -14.5--0.2)
Harm	No description	No adverse events	No adverse events
Conclusion	Dynamic balance exercises on fixed and compliant sponge surfaces were feasibly coupled to interactive video game-based exercise. This coupling, in turn, resulted in a greater improvement in dynamic standing balance control compared with the typical exercise program. However, there was no transfer of effect to gait function	The results suggest that eBaViR represents a safe and effective alternative to traditional treatment to improve static balance in the ABI population	Virtual reality Wii gaming technology represents a safe, feasible, and potentially effective alternative to facilitate rehabilitation therapy and promote motor recovery after stroke
Trial registration	Clinical Trials.gov (NCT01381237)	No registration	No description
Found	Grant from the Riverview Health Centre Foundation, Winnipeg, Manitoba, Canada: The Fund provided the space at their facility and access to their day hospital program clients for assessment and treatment of the control group	Ministerio de Educación y Ciencia Spain, Projects Consolider-C (SEJ2006-14301/ PSIC), "CIBER of Physiopathology of Obesity and Nutrition, an initiative of ISCIII" and the Excellence Research Program PROMETEO	This study was supported by a grant from the Ministry of Health and Long Term Care through the Ontario Stroke System, administered by Heart and Stroke Foundation of Ontario
Cost of intervention	No description	No description	No description
Ref.	Bauer *et al*[30]	Adamo *et al*[31]	Hackney *et al*[32]
Citation	*J Womens Health* (Larchmt) 2010; 19: 523-531	*Appl Physiol Nutr Metab* 2010; 35: 805-815	*J Rehabil Med* 2009; 41: 475-481
Title	Alleviating distress during antepartum hospitalization: a randomized controlled trial of music and recreation therapy	Effects of interactive video game cycling on overweight and obese adolescent health	Effects of dance on movement control in PD: a comparison of Argentine tango and American ballroom
Aim/objective	To examine the efficacy of a single session music or recreation therapy intervention to reduce antepartum-related distress among women with high-risk pregnancies extended antepartum hospitalizations	To examine the efficacy of interactive video game stationary cycling (GameBike) in comparison with stationary cycling to music on adherence, energy expenditure measures, submaximal aerobic fitness, body composition, and cardiovascular disease risk markers in overweight and obese adolescents, using a randomized controlled trial design	To compare the effects of tango, waltz/foxtrot and no intervention on functional motor control in individuals with PD
Setting/place	Midwestern, suburban teaching hospital with a regional Perinatal Center with 26 private rooms on the antepartum unit	The Endocrinology clinic at the Children's Hospital of Eastern Ontario	No description
Participants	Participants (n = 80) were hospitalized with various high-risk obstetric health issues, including preterm labor, premature rupture of membranes, preeclampsia, and multiple gestations. They were all over the age of 18 (mean age 31 yr), between 24 and 38 wk of gestation	Thirty obese adolescents between ages of 12-17 yr	Fifty-eight participants with idiopathic PD participated. They were at least 40 yr of age, could stand for at least 30 min, and walk independently for ≥ 3 m with or without an assistive device

Intervention	Participants were received a 1-h music or recreation therapy intervention. Music therapists offered a range of interventions for patients, all within the current standards of care of these therapies, included music-facilitated relaxation, active music listening, song writing, music for bonding, and clinical improvisation. Recreation therapy interventions offered included adaptive leisure activities, creative arts, community resource education, and leisure awareness activities	In the experimental group (interactive video game cycling), participants (n = 15) were required to exercise on a GameBike interactive video gaming system that was interfaced with a Sony Play Station 2. Participants were allowed to select from variety of choices, video games to play while cycling and were permitted to switch games during the exercise session. In control group (stationary cycling to music), participants were allowed to listen to music of their choice *via* radio, CD, or personal music device. The instructions given participants and the general protocol for this condition was the same as for video game condition. The 10-wk program consisted of twice weekly sessions lasting a maximum of 60 min per session, respectively	The both dance classes were taught by the same instructor who was an experienced professional ballroom dance instructor and an American Council on Exercise certified personal trainer. Those in the dance groups attended 1-h classes twice a week, completing 20 lessons in 13 wk. Both genders spent equal time in leading and following dance roles. Healthy young volunteers, recruited from physical therapy, pre-physical therapy and pre-medical programs at Washington University and St. Louis University, served as dance partners for those with PD. Volunteers were educated about posture and gait problems associated with PD
Main and secondary outcomes	Antepartum Bedrest Emotional Impact Inventory Scores	Adherence, submaximal aerobic fitness (Peak workload,Time to exhaustion,Peak heart rate), exercise behaviour, body composition, and blood parameters	The Unified Parkinson's Disease Rating Scale Motor Subscale 3 (UPDRS), BBS, TUG, 6MWT, FOG questionnaire, and forward and backward gait (gait velocity, stride length, and single support time)
Randomisation	The groups were assigned by the research coordinator (using a Random Numbers Statistical Table and opaque envelopes containing group membership) to an intervention condition (either a music or recreation therapy) or waitlist control condition	The randomization schedule was computer generated using a basic random number generator	Randomly selecting one of the 3 conditions from a hat
Blinding/masking	Only participants were blinded (single blinding)	No blinding	The first author was not blinded to group assignment. The evaluations were videotaped for a rater who was a specially trained physiotherapy student otherwise not involved in the study (blinded assessor). Participants were not informed of the study hypotheses
Numbers randomised	Music therapy group (n = 19), recreation therapy group (n = 19), and control group (n = 42)	Video game cycling (n = 15) and Music cycling (n = 15)	Waltz/foxtrot (n = 19), Tango (n = 19), and Control (n = 20)
Recruitment	Identified eligible patients through chart review and nursing report during 2003-2005. A total of 136 patients; once enrolled, however, 56 patients were unable to complete the study	Participants were recruited between May 2007 and January 2009 and the final subject assessment was completed in March 2009. A total of 150 families were screened through the Endocrinology clinic at the Children's Hospital of Eastern Ontario to determine Assessed for eligibility. Thirty families me the all inclusion criteria	Participates were recruited from the St. Louis community through advertisement at local support groups and local community events. Most were directly recruited *via* telephone from the Washington University Movement Disorders Center database
Numbers analysed	Music therapy group (n = 19), recreation therapy group (n = 19), and control group (n = 42)	Video game cycling (n = 13) and Music cycling (n = 13)	Waltz/foxtrot (n = 17), Tango (n = 14), and Control (n = 17)
Outcome	Significant association were found between the delivery of music and recreation therapy and reduction of antepartum-related distress in women hospitalized with high-risk pregnancies. These statistically significant reductions in distress persisted over a period of up to 48-72 h (each P < 0.05)	The music group had a higher rate of attendance compared with the video game group (92% *vs* 86%, P < 0.05). Time spent in minutes per session at vigorous intensity (80%-100% of predicted peak heart rate) (24.9 ± 20 min *vs* 13.7 ± 12.8 min, P < 0.05) and average distance (km) pedaled per session (12.5 ± 2.8 km *vs* 10.2 ± 2.2 km, P < 0.05) also favoured the music group. However, both interventions produced significant improvements in submaximal indicators of aerobic fitness as measured by a graded cycle ergometer protocol	Significant improvements were noted in tango and waltz/foxtrot on the BBS, 6MWT and backward stride length when compared with controls (P < 0.05). Control group worsened significantly with respect to disease severity, as measured by the UPDRS, and on time spent in single support during forward and backward walking
Harm	No description	No adverse events	No description

Conclusion	Single session music and recreation therapy interventions effectively alleviate antepartum-related distress among high-risk women experiencing antepartum hospitalization and should be considered as valuable additions to any comprehensive antepartum program	The results supported the superiority of cycling to music and indicated investing in the more expensive GameBike may not be worth the cost	Tango may target deficits associated with PD more than waltz/foxtrot, but both dances may benefit balance and locomotion
Trial registration	No description	Clinical Trials.gov (NCT00983970)	No description
Found	No description	The Canadian Diabetes Association	The American Parkinson's Disease Association and NIH grant K01-048437
Cost of intervention	No description	Participants and their families were reimbursed CAN$10 per visit to the laboratory for parking and transportation costs, and the participants were given a CAN$20 movie theatre gift certificate following trial completion	No description
Ref.	Yavuzer *et al*[33]	Desrosiers *et al*[34]	Siedliecki *et al*[4]
Citation	*Eur J Phys Rehabil Med* 2008; 44: 237-244	*Arch Phys Med Rehabil* 2007; 88: 1095-1100	*J Adv Nurs* 2006; 54: 553-562
Title	''Playstation eyetoy games'' improve upper extremity-related motor functioning in subacute stroke: a randomized controlled clinical trial	Effect of a home leisure education program after stroke: a randomized controlled trial	Effect of music on power, pain, depression and disability
Aim/objective	To evaluate the effects of "Playstation EyeToy games" on upper extremity motor recovery and upper extremity-related motor functioning of patients with subacute stroke	To evaluate the effect of a leisure education program on participation in and satisfaction with leisure activities (leisure-related outcomes), and well-being, depressive symptoms, and quality of life (primary outcomes) after stroke	To test the effect of music levels of power, pain, depression, and disability; to compare the effect of researcher-provided relaxing music choices with subject-preferred music, selected daily based on self-assessment; and to test the relationship between power and the combined dependent variable of pain, depression and disability
Setting/place	Twenty inpatients with hemiparesis after stroke in rehabilitation center from the general hospital, Turkey	Home and community	Pain clinics and chiropractic office in northeast Ohio, United States
Participants	Twenty hemiparetic inpatients with post-stroke. Eligible criteria: (1) first hemiparesis within 12 mo; (2) Brunnstrom stage 1-4 for upper extremity; and (3) no severe cognitive disorders	Sixty-two people (mean age 70 yr) with stroke	Participation of 60 African American and Caucasian people aged 21-65 yr (mean age 49.7 yr) with chronic non-malignant pain CNMP
Intervention	Both the intervention group and the control group participated in a conventional stroke rehabilitation program, 5 d a week, 2-5 h/d for 4 wk. The conventional program is patient-specific and consists of neurodevelopmental facilitation techniques, physiotherapy, OT, and speech therapy. For the same 4-wk of period, the EyeToy group received an additional 30 min of VR therapy program	The experimental participants (n = 33) received the leisure education program (leisure awareness, self-awareness, and competence development) at home once a week for 8 to 12 wk. The recreational therapist was responsible for the intervention whereas the occupational therapist acted as a consultant. The control participants (n = 29) were also visited by the recreation therapist but the topics discussed were unrelated to leisure (*e.g.*, family, cooking, politics, news, everyday life)	Patterning Music (PM; subject-preferred music) group were asked to select upbeat, familiar, instrumental or vocal music to ease muscle tension and stiffness. Standard Music (SM; researcher-provided music) group were offered a choice of one 60-min relaxing instrumental music tape from a collection of five tapes (piano, jazz, orchestra, harp and synthesizer) used in several music and acute pain studies. Each group received their assigned intervention for 1-h a day for 7 consecutive days. Control group received standard care that did not include music intervention, and all participants kept a diary for 7 d
Main and secondary outcomes	Brunnstrom stages and FIM	Minutes of leisure activity per day, number of leisure activities, the Leisure Satisfaction Scale, the Individualized Leisure Profile, the GWBS, the Center for Epidemiological Studies Depression Scale, and the SA-SIP30	Power (characterize power: awareness, choices, freedom, and a personal involvement in creating change), pain, depression, and disability
Randomisation	The randomization schedule was computer generated using a basic random number generator	The randomization schedule was computer generated using a basic random number generator	The random allocation sequence using the Min-8 program
Blinding/masking	Assessor was blinded to the group allocation of the subject. Patients and physical therapist were not blinded	Only assessor was blinded	No description
Numbers randomised	Intervention group (n = 10) and Control group (n = 10)	Experimental participants (n = 33) and Control participants (n = 29)	PM group (n = 18), SM group (n = 22), and Control group (n = 20)

Recruitment	"Inpatients with hemiparesis after stroke"	A total of 62 people entered the trial carried out in 2002 and 2003. Authors recruited them after a review of medical charts of people (n = 230) who were previously admitted with stroke to a rehabilitation or acute care facility up to 5 yr before the study	64 patients with CNMP was recruited over a 24-mo period from 2001 to 2003 from pain clinics and a chiropractic office in northeast Ohio
Numbers analysed	Intervention group (n = 10) and Control group (n = 10)	Experimental participants (n = 29) and Control participants (n = 27)	PM group (n = 18), SM group (n = 22), and Control group (n = 20),
Outcome	The mean change score (95%CI) of the FIM self-care score [(5.5 (2.9-8.0) *vs* 1.8 (0.1-3.7), P = 0.018] showed significantly more improvement in the EyeToy group compared to the control group. No significant differences were found between the groups for the Brunnstrom stages for hand and upper extremity	There was a statistically significant difference in change scores between the groups for satisfaction with leisure with a mean difference of 11.9 points (95%CI: 4.2-19.5) and participation in active leisure with a mean difference of 14.0 min (95%CI: 3.2-24.9). There was also a statistically significant difference between groups for improvement in depressive symptoms with a mean difference of -7.2 (95%CI: -12.5--1.9). Differences between groups were not statistically significant on the SA-SIP30 (0.2; 95%CI: -1.3-1.8) and GWBS (2.2; 95%CI: -5.6-10.0)	The music groups had more power and less pain (P = 0.002), depression (P = 0.001) and disability (P = 0.024) than the control group, but there were no statistically significant differences between the two music interventions. The model predicting both a direct and indirect effect for music was supported
Harm	No adverse events	No description	No description
Conclusion	"Playstation EyeToy Games" combined with a conventional stroke rehabilitation program have a potential to enhance upper extremity-related motor functioning in subacute stroke patients	The results indicate the effectiveness of the leisure education program for improving participation in leisure activities, improving satisfaction with leisure and reducing depression in people with stroke	Nurses can help patients with CNMP identify and use music they enjoy as a self-administered complementary intervention to facilitate feelings of power, and to decrease perceptions of pain, depression and disability
Trial registration	No description	No description	No description
Found	No description	The Canadian Institutes of Health Research (MOP-49526)	The Frances Payne Bolton Alumni Association, Case Western Reserve University, Cleveland Ohio; Sigma Theta Tau, Delta Omega Research Grant; NRSA (NINR; NIH#1F31nro7565)
Cost of intervention	No description	No description	No description
Ref.	Fitzsimmons[5]	Parker *et al*[6]	
Citation	*J Gerontol Nurs* 2001; 27: 14-23	*Clin Rehabil* 2001; 15: 42-52	
Title	Easy rider wheelchair biking. A nursing-recreation therapy clinical trial for the treatment of depression	A multicentre randomized controlled trial of leisure therapy and conventional occupational therapy after stroke. TOTAL Study Group. Trial of Occupational Therapy and Leisure	
Aim/objective	To determine if participation in a therapy biking program had an effect on the degree of depression in older adults living in a long-term facility in upstate New York	To evaluate the effects of leisure therapy and conventional OT on the mood, leisure participation and independence in ADL of stroke patients 6 and 12 mo after hospital discharge	
Setting/place	The New York State Home for Veterans (Veterans' Home)	Five UK centres: Aintree Fazakerley Hospital, Bristol Southmead Hospital, Edinburgh Western General Hospital, Glasgow Royal Infirmary and Nottingham University Hospital	
Participants	Thirty-nine older adults (mean age 80 yr) with depression living a long-term facility	Four hundred and sixty-six stroke patients (mean age 72 yr)	
Intervention	Ease rider Program (Therapy program) intervention. The experimental groups received the therapeutic biking program for 1 h a day, 5 d a week, for 2 wk	Two treatment groups (ADL group and Lisure group) received OT interventions at home for up to 6 mo after recruitment. The protocol specified a minimum of 10 sessions lasting not less than 30 min each. The treatment goals set in the ADL group were in term of improving independence in self-care tasks and therefore treatment involved practising these task (such as preparing a meal or walking outdoor). For the leisure group, goals were set in term of leisure activity and so interventions included practising the leisure task as well as any ADL tasks necessary achieve the leisure objective. Control group received no OT treatment within the trial	

Main and secondary outcomes	The short-form Geriatric Depression Scale	For mood, the GHQ/For leisure activity, the Nottingham Leisure Questionnaire/For independence in ADL, the Nottingham Extended ADL Scale
Randomisation	No description	The Collaborative Stroke Audit and Research telephone randomization service was used to allocate patients to one of three group: leisure, ADL and control
Blinding/masking	No description	Only participants were blinded
Numbers randomised	Treatment group (n = 20) and Control group (n = 20)	Leisure group (n = 153), ADL group (n = 156), and Control group (n = 157)
Recruitment	The target population (n = 90) was residents with a diagnosis of or symptoms of depression in the New York State Home for Veterans	Recruitment was conducted at five UK centres: Aintree Fazakerley Hospital, Bristol Southmead Hospital, Edinburgh Western General Hospital, Glasgow Royal Infirmary and Nottingham University Hospital. 1750 patients was registered
Numbers analysed	Treatment group (n = 19) and Control group (n = 20)	Leisure group (n = 113), ADL group (n = 106), and Control group (n = 112)
Outcome	The control groups' GDS pretest means of 7.95 increased slightly at the posttest to 8.65, indicating a slight increase (+0.70) in depression. The treatment groups' pretest 7.68 decreased to 4.21 (-3.47) at the posttest, denoting a marked decrease in depression (P < 0.001)	At 6 mo and compared to the control group, those allocated to leisure therapy had nonsignificantly better GHQ scores (-1.2: 95%CI: -2.9-0.5), leisure scores (+0.7: 95%CI: -1.1-2.5) and Extended ADL scores (+0.4: 95%CI: -3.8-4.5): the ADL group had nonsignificantly better GHQ scores (-0.1: 95%CI: -1.8-1.7) and Extended ADL scores (-1.4: 95%CI: -2.9-5.6) and nonsignificantly worse leisure scores (-0.3: 95%CI: -2.1-1.6). The results at 12 mo were similar
Harm	No adverse events	No description
Conclusion	This study contributes to the body of knowledge of nursing regarding options for the treatment of depression in older adults, and is an encouraging that psychosocial interventions may be effective in reducing depression	In contrast to the findings of previous smaller trials, neither of the additional OT treatments showed a clear beneficial effect on mood, leisure activity or independence in ADL measured at 6 or 12 mo
Trial registration	No description	No description
Found	The NewYork State Dementia Research Grant 2000	NHS Research and Development Programme
Cost of intervention	The cost of a basic bike is approximately $3600 plus shiping	No description

ABI: Acquired brain injury; PD: Parkinson's disease; BBS: Berg Balance Scale; RT: Recreational therapy; MMSE: Mini-Mental State Examination; TUG: Timed "Up and Go" Test; ABC: Activities-specific Balance Condidence Scale; ART: Anterior Reach Test; 6MWT: 6-min walk test; CNMP: Chronic non-malignant pain; OT: Occupational therapy; GWBS: General Well-Being Schedule; SA-SIP30: Stroke-Adapted Sickness Impact Profile; FIM: Functional Independence Measure; ADL: Activities of daily living; GHQ: General Health Questionnaire.

(11%); "results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory" (14%); "all important harmful or unintended effects in each group" (27%); "generalizability (external validity) of the trial findings according to the intervention, comparators, patients, and care providers and centers involved in the trial" (27%); "registration number and name of trial registry" (18%); and "where the full trial protocol can be accessed, if available" (18%).

Meta-analysis of balance ability

Results from RCTs with control groups[27,28] were pooled in a meta-analysis to establish the overall effect of balance ability interventions compared with no-interventions controls (Figures 2 and 3). For the Berg Balance Scale (BBS), the included interventions were sufficiently homogenous (I^2 = 62.8%, P = 0.101), so the fixed effects model was used. This revealed a non-significant difference in balance ability favoring interventions over controls at the last reported assessment (SMD = 3.75; 95%CI: 1.82-5.69; n = 44). For the Timed "Up and Go" (TUG), the interventions were homogenous (I^2 = 69.5%, P = 0.070), so the fixed effects model was also used. This revealed a no significant difference in balance ability favoring interventions over controls (SMD = 0.19; 95%CI: -4.09-4.47; n = 44). A funnel plot to assess publication bias was not generated as fewer than 10 interventions were included in the meta-analysis[35].

Withdrawals and adverse events

Five studies[5,28,29,31,33] reported no adverse events during all interventions but there were no descriptions of adverse events in the other studies (Table 3). Two stud-

Table 4 Evaluation of the quality of randomized controlled trials by using the CONSORT 2010 checklist and the checklist for reporting trials nonpharmacologic treatments

Paper Section/ Topic	ID	CONSORT 2010; items	Checklist for reporting trials of nonpharmacologic treatment: items	Ref.											Present description[1]	
				[27]	[28]	[29]	[30]	[31]	[32]	[33]	[34]	[4]	[5]	[6]	No/sum	Rate (%)
Title and abstract	1a	Identification as a randomised trial in the title		p	p	p	p	a	?	p	p	a	a	p	7/11	64
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)		n/a	n/a	p	p	p	p	p	p	?	?	p	7/9	78
			In the abstract, description of the experimental treatment, comparator, care providers, centers, and blinding status	p	p	p	?	?	?	?	p	?	?	?	4/11	36
Introduction																
Background and objectives	2a	Scientific background and explanation of rationale		p	p	p	p	p	p	?	p	p	p	p	10/11	91
	2b	Specific objectives or hypotheses		p	p	p	p	p	p	p	p	p	p	p	11/11	100
Methods																
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio		p	?	p	p	p	p	p	p	p	p	p	10/11	91
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons		p	p	?	p	a	?	p	a	a	a	a	4/11	36
Participants	4a	Eligibility criteria for participants		p	p	p	p	p	p	p	p	p	p	p	11/11	100
	4b	Settings and locations where the data were collected	When applicable, eligibility criteria for centers and those performing the interventions	p	?	p	p	p	?	p	p	p	p	p	9/11	82
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Precise details of both the experimental treatment and comparator	p	p	p	p	?	p	p	?	p	p	p	9/11	82
			Description of the different components of the interventions and, when applicable, descriptions of the procedure for tailoring the interventions to individual participants	a	a	p	p	a	p	a	a	p	p	p	6/11	55
			Details of how the interventions were standardized	a	a	a	p	a	p	n/a	a	p	?	p	4/10	40
			Details of how adherence of care providers with the protocol was assessed or enhanced	a	?	a	n/a	a	p	n/a	a	?	?	?	1/9	11
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed		p	p	p	p	p	p	p	p	?	?	p	9/11	82
	6b	Any changes to trial outcomes after the trial commenced, with reasons		p	p	a	n/a	a	n/a	n/a	a	a	a	a	2/8	25
Sample size	7a	how sample size was determined		p	p	a	?	a	?	p	a	a	p	p	5/11	45
	7b	when applicable, explanation of any interim analyses and stopping guidelines		p	a	a	n/a	a	n/a	?	a	a	p	p	3/9	33
			When applicable, details of whether and how the clustering by care providers or centers was addressed	p	a	a	n/a	a	n/a	?	a	?	?	p	2/9	22
Randomisation: Sequence generation	8a	Method used to generate the random allocation sequence		?	p	?	p	p	p	p	p	p	a	p	8/11	73

	8b	Type of randomisation; details of any restriction (such as blocking and block size)		n/a	n/a	p	n/a	?	n/a	p	?	a	a	a	2/7	29
			When applicable, how care providers were allocated to each trial group	n/a	n/a	a	n/a	?	n/a	a	?	p	?	p	2/7	29
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned		a	a	p	p	?	p	p	?	p	a	p	6/11	55
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions		a	a	p	p	?	p	p	?	a	a	p	5/11	45
			Details of the experimental treatment and comparator as they were implemented	a	a	p	p	?	p	p	?	p	?	p	6/11	55
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how		p	p	p	p	a	p	p	p	a	a	a	7/11	64
			Whether or not those administering co-interventions were blinded to group assignment	?	?	a	?	a	?	a	?	a	a	a	0/11	0
	11b	if relevant, description of the similarity of interventions	If blinded, method of blinding and description of the similarity of interventionist	?	?	a	p	a	p	a	a	a	a	a	2/11	18
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes		?	?	p	p	p	p	p	p	p	p	p	9/11	82
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses		a	a	n/a	n/a	a	n/a	p	a	p	a	p	3/8	38
			when applicable, details of whether and how the clustering by care providers or centers was addressed	a	a	n/a	n/a	a	n/a	a	a	p	p	p	3/8	38
Results																
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	The number of care providers or centers performing the intervention in each group and the number of patients treated by each care provider or in each center	a	a	p	p	p	?	p	p	p	p	p	8/11	73
	13b	For each group, losses and exclusions after randomisation, together with reasons		?	?	p	p	p	p	p	p	p	p	p	9/11	82
Recruitment	14a	Dates defining the periods of recruitment and follow-up		?	?	p	?	p	?	p	p	p	p	p	7/11	64
	14b	Why the trial ended or was stopped		?	?	p	n/a	p	n/a	p	p	p	a	p	6/9	67
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	When applicable, a description of care providers (case volume, qualification, expertise, *etc*.) and centers (volume) in each group	p	p	p	p	a	p	p	a	a	a	p	7/11	64
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups		p	p	p	p	p	p	p	p	p	p	p	11/11	100
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)		p	p	p	p	a	p	p	p	p	p	p	10/11	91

	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	?	?	p	n/a	a	n/a	a	a	a	a	a	1/9	11
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing prespecified from exploratory	a	a	n/a	n/a	a	n/a	n/a	a	p	a	a	1/7	14
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	a	p	a	?	p	?	p	a	a	a	a	3/11	27
Discussion															
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	p	p	p	p	a	p	p	p	p	p	a	9/11	82
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	p	p	p	p	?	p	?	?	p	a	a	6/11	55
		In addition, take into account the choice of the comparator, lack of or partial blinding, and unequal expertise of care provides or centers in each group													
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	p	p	p	p	?	p	p	?	p	?	p	8/11	73
		Generalizability (external validity) of the trial findings according to the intervention, comparators, patients, and care providers and centers involved in the trial	?	?	p	p	?	?	?	?	p	?	?	3/11	27
Other information															
Registration	23	Registration number and name of trial registry	p	a	?	?	p	?	?	?	?	?	?	2/11	18
Protocol	24	Where the full trial protocol can be accessed, if available	p	a	p	?	a	?	a	a	a	a	a	2/11	18
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	p	?	p	?	a	p	p	p	p	p	p	8/11	73

[1]Present description means present No. and sum except "n/a" and its percentage. a: Absent; ?: Unclear or inadequately described.

ies[29,32] reported no withdrawals (dropouts), nine studies showed some dropouts because of mainly death, death in family, hospitalization, and injuries due to other causes. The reasons preventing patients from recreational activities were not shown.

Costs of intervention

Two studies[5,31] described the costs of intervention (Table 3). Adamo *et al*[31] showed parking and transportation costs, as well as movie theatre gift certificates following the trial completion. Fitzsimmons[5] showed the cost of an easy rider wheelchair bike. There was no information regarding costs of intervention in the other studies.

DISCUSSION

This is the first SR of the effectiveness of rehabilitation based on recreational activities. Eleven RCTs were identified, target diseases and/or symptoms included stroke, dementia, Parkinson's disease, acquired brain injury, CNMP, adolescent obesity, high-risk pregnancy, and the frail elderly. The intervention methods included various approaches such as gaming technology, music, dance, easy rider wheelchair biking, leisure education programs, and leisure tasks. Primary or secondary outcomes were generally psychological status

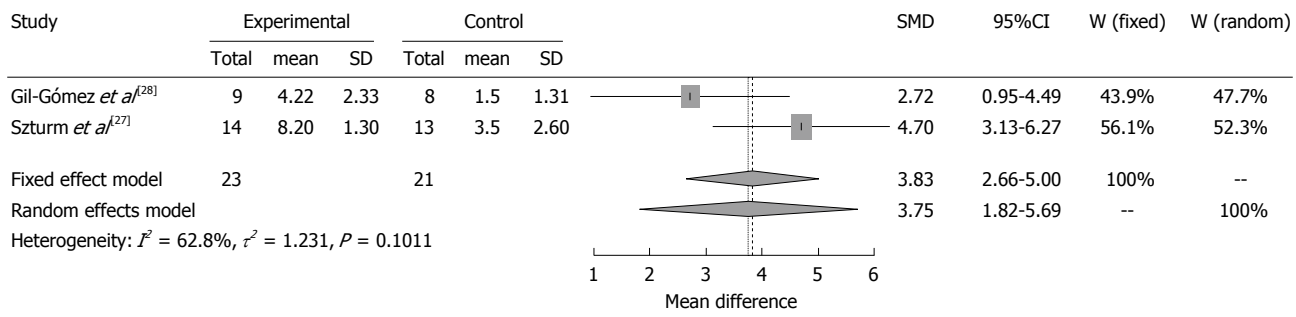

Figure 2 A meta-analysis on the effect of the Berg Balance Scale by gaming intervention. SMD: Standardized mean difference.

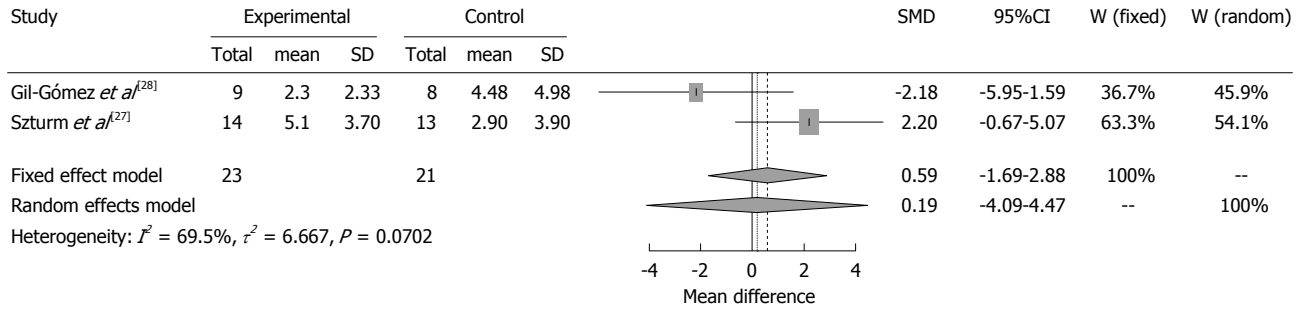

Figure 3 A meta-analysis on the effect of the Timed "Up and Go" Test by gaming intervention. SMD: Standardized mean difference.

(depression, mood, emotion, and power), balance or motor function, and adherence (feasibility and attendance).

Video gaming as new trend of rehabilitation

The trend over the past 10 years towards game interventions by VR is particularly interesting. Basically, sedentary screen time has been shown to be associated with obesity[36] as well as negative health outcomes such as premature death[37,38], independent of physical activity levels[39]. However, one strategy, the term "active video gaming" or "virtual gaming" has been used to describe games in which body movement is necessary or encouraged by the control scheme of each game. Typically, active games use a motion-sensing or motion-encouraging controller rather than a traditional handheld game pad controller. Lyons *et al*[40] reported that dance simulation and fitness games seemed to have the potential to produce moderate-intensity physical activity in physiological experiments. A recent SR[41] without meta-analysis, based on video games reported that there is potential for video games to improve health-related outcomes, particularly in the area of psychological and physical therapy. However, the included RCTs were of relatively low quality. A discussion, including a meta-analysis to clearly demonstrate an effect of the video game, was required.

Meta-analysis of balance ability based on video games

For BBS and TUG as an indicator of balance ability, the interventions were not identical, but the results for both revealed no significant differences in balance ability between interventions and controls. One reason for this was that the pooled sample size was very small (two studies, 44 participants) and we could not, therefore, calculate and describe a funnel plot to assess publication bias. It may be difficult to recruit many patients as participants in rehabilitation studies, although studies (cluster- or multicenter-RCTs depending on the case) with sufficient numbers of subjects are necessary. The second problem may be that the dose-regimen, such as period and frequency of the interventions, was inadequate. The mental and physical burden on participants is increased when there is substantial intervention, although it is expected that the effect of balance ability would rise in a positive relationship with the quantity of intervention. Because a gradual increase in load with recovery is necessary in rehabilitation programs, it is easy to assign settings like "Level" or "Stage" for the game, such as first, second level, *etc*. Therefore, we also expect to understand correctly the results and detailed descriptions of "pragmatic trials"[42] as well as "explanatory trials" for the rehabilitation effects of game intervention.

Non-meta-analysis of other recreation activities

In all other interventions, there wa at least one effect on psychological status, balance or motor function, and adherence as the primary or secondary outcomes. However, it was impossible to perform a meta-analysis and integrate the results since the main outcome measures and interventions were different. Therefore, we recognize the potential for recreational activities to improve rehabilitation effects, but could not provide conclusive evidence of these rehabilitation effects.

Overall evidence and quality assessment

The CONSORT 2010 and the CONSORT for non-phar-

Table 5 Overall evidence and future research agenda to build evidence

Overall evidence in the present	Research agenda
There is potential for effects such as psychological status, balance or motor function, and adherence but overall evidence remains unclear	Structural description of papers based on the CONSORT 2010 and the CONSORT for nonpharmacological trials 1 Satisfactory description and methodology (Method used to generate the random allocation sequence, blinding, care provider, estimated effect size and its precision, harm, external validity, and trial registry with protocol) 2 Description of intervention dose (if pragmatic intervention) 3 Adequate sample size to perform a meta-analysis 4 Description of adverse effects (*e.g.*, dizziness by watching screen) 5 Description of withdrawals 6 Description of cost (*e.g.*, gaming equipment) 7 Development of the original check item in recreation activity

macological trials checklists were not originally developed for use as quality assessment instruments, but we used them as such because they are the most important tools related to the internal and external validity of trials. There were serious problems with the conduct and reporting of the target studies. In particular, our review detected omissions in the following descriptions: methods used to generate the random allocation sequence, blinding, care provider, estimated effect size and its precision, harm, external validity, and trial registry with protocol. Descriptions of these items were lacking (very poor; < 50%) in many studies.

In the Cochrane Review, the eligibility criteria for a meta-analysis are strict, and for each article, heterogeneity and low quality of reporting must first be excluded. Because there was insufficient evidence in the studies of recreational intervention, due to poor methodological and reporting quality as well as heterogeneity, we are unable to offer any conclusions about the effects of rehabilitation by recreational intervention based on RCTs. Both the CONSORT 2010 and the CONSORT for nonpharmacological trials checklists are relatively new, but it was shown that the study protocol description and implementation for recreational studies could be subjected to these checklists.

Overall evidence and future research agenda to build evidence

The results of this study suggest that few RCTs have been conducted in this area, and that the RCTs conducted have been of relatively low quality. Table 5 shows the future research agenda for studies of the rehabilitation effect by recreational activity. There is potential for effects on psychological status, balance or motor function, and adherence, but the overall evidence remains unclear. Therefore, researchers should use the appropriate checklists for research design and intervention method, as this would lead to improvement in the quality of the study, and would contribute to the accumulation of evidence. Researchers should also present not only the efficacy data, but also description of any adverse events or harmful phenomena and withdrawals. Many studies in this review did not describe these factors.

A recent study[43] suggested that public health is moving toward the goal of implementing evidence based intervention. However, the feasibility of possible interventions and whether comprehensive and multilevel evaluations are needed to justify them must be determined. It is at least necessary to show the cost of such interventions. We must choose to introduce an interventional method based on its cost-benefit, cost-effectiveness, and cost-utility. In addition, recreational activities as intervention are unique and completely different than pharmacological or traditional rehabilitation methods. Therefore it may be necessary to add some original items such as herbal intervention[44], aquatic exercise[45], and balneotherapy[46] to the CONSORT checklist as alternative or complementary medicines.

Strength and limitations

This review had several strengths: (1) the methods and implementation registered high on the PROSPERO database; (2) it was a comprehensive search strategy across multiple databases with no data restrictions; (3) there were high agreement levels for quality assessment of articles; and (4) it involved detailed data extraction to allow for collecting all of an article's content into a recommended structured abstract. The conduct and reporting of this review also aligned with the PRISMA statement[47] for transparent reporting of SRs and meta-analyses.

This review also had several limitations that should be acknowledged. Firstly, although some selection criteria were common across studies, as described above, bias remained due to differences in eligibility for participation in each study. Secondly, publication bias was a limitation. Although there was no linguistic restriction in the eligibility criteria, we searched studies with only English and Japanese key words. In addition, this review reported on a relatively small and heterogeneous sample of studies. Moreover we could not follow standard procedures for estimating the effects of moderating variables. Finally, although we used an original definition of recreation activity because of the lack of a clear worldwide definition, our definition was not universal.

In conclusion, this comprehensive SR demonstrates that recreational activities may have the potential for im-

proving rehabilitation in a wide variety of areas, and for a variety of patients and elderly people. To most effectively assess the potential benefits of recreational activities for rehabilitation, it will be important for further research to utilize (1) RCT methodology (person unit or cluster unit) when appropriate; (2) an intervention dose; (3) a description of adverse effects and withdrawals; and (4) the cost of recreation activities.

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COMMENTS

Background

Recreational activity is anything that is stimulating and rejuvenating for an individual. "Enjoyment" is an important factor that may aid adherence to training for rehabilitation.

Research frontiers

Although many studies have reported the rehabilitation effects of recreational activities, there is no systematic review (SR) of the evidence based on randomized controlled trials.

Innovations and breakthroughs

This is the first SR of the effectiveness of rehabilitation based on recreational activities. There were serious problems with the conduct and reporting of the target studies. In particular, this review detected omissions in the following descriptions: methods used to generate the random allocation sequence, blinding, care provider, estimated effect size and its precision, harm, external validity, and trial registry with protocol. Descriptions of these items were lacking (very poor; < 50%) in many studies.

Applications

There is a potential for recreational activities to improve rehabilitation-related outcomes, particularly in psychological status (depression, mood, emotion, and power), balance or motor function, and adherence (feasibility and attendance).

Terminology

For rehabilitation, the World Health Organization explains that rehabilitation of people with disabilities is a process aimed at enabling them to reach and maintain their optimal physical, sensory, intellectual, psychological and social functional levels. The definition of the recreational activity is complex but, in this study, it distinguishes the specific exercise item. Specifically, any kind of recreation activity (not only dynamic activities but also musical appreciation or play, painting, hand-craft, *etc.*) was permitted and defined as an intervention.

Peer review

The authors have done an excellent job in presenting results, with a format different than that normally employed in works of meta-analysis. This did not include the usual estimates of effect size based on meta-analytical indicators but is likely that this did not lead to major complications, given the number of studies analyzed. It is a good descriptive work, very systematic and ordered.

REFERENCES

1 **Pan W**. Examples of Recreational Activities - Fun Things to Do. Available from: URL: http://ezinearticles.com/?Examples-of-Recreational-Activities---Fun-Things-to-Do&id=1566968. Accessed July 29, 2012
2 **Veal AJ**. Research methods for leisure and tourism: a practical guide. London: Pearson Education, 2006
3 **Kamioka H**, Tsutani K, Takahashi M, Honda T, Moriyama S, Mutoh Y, Yamada Y, Makishi M, Shimojima H. A systematic review of randomized controlled trials concerning leisure activity and recreation. *J Leisure Recreation Studies* 2008; **60**: 29-37
4 **Siedliecki SL**, Good M. Effect of music on power, pain, depression and disability. *J Adv Nurs* 2006; **54**: 553-562 [PMID: 16722953 DOI: 10.1111/j.1365-2648.2006.03860.x]
5 **Fitzsimmons S**. Easy rider wheelchair biking. A nursing-recreation therapy clinical trial for the treatment of depression. *J Gerontol Nurs* 2001; **27**: 14-23 [PMID: 11915269]
6 **Parker CJ**, Gladman JR, Drummond AE, Dewey ME, Lincoln NB, Barer D, Logan PA, Radford KA. A multicentre randomized controlled trial of leisure therapy and conventional occupational therapy after stroke. TOTAL Study Group. Trial of Occupational Therapy and Leisure. *Clin Rehabil* 2001; **15**: 42-52 [PMID: 11237160 DOI: 10.1191/026921501666968247]
7 **Donnan GA**, Fisher M, Macleod M, Davis SM. Stroke. *Lancet* 2008; **371**: 1612-1623 [PMID: 18468545 DOI: 10.1016/S0140-6736(08)60694-7]
8 **Mayo NE**, Wood-Dauphinee S, Ahmed S, Gordon C, Higgins J, McEwen S, Salbach N. Disablement following stroke. *Disabil Rehabil* 1999; **21**: 258-268 [PMID: 10381238]
9 **French B**, Thomas LH, Leathley MJ, Sutton CJ, McAdam J, Forster A, Langhorne P, Price CI, Walker A, Watkins CL. Repetitive task training for improving functional ability after stroke. *Cochrane Database Syst Rev* 2007; CD006073 [PMID: 17943883 DOI: 10.1002/14651858]
10 **Laver KE**, George S, Thomas S, Deutsch JE, Crotty M. Virtual reality for stroke rehabilitation. *Cochrane Database Syst Rev* 2011; CD008349 [PMID: 21901720 DOI: 10.1002/14651858.CD008349.pub2]
11 **Griffin M**, McCormick D, Taylor MJ, Shawis T, Impson R. Using the Nintendo Wii as an intervention in a falls prevention group. *J Am Geriatr Soc* 2012; **60**: 385-387 [PMID: 22332691 DOI: 10.1111/j.1532-5415.2011.03803.x]
12 **Padala KP**, Padala PR, Burke WJ. Wii-Fit as an adjunct for mild cognitive impairment: clinical perspectives. *J Am Geriatr Soc* 2011; **59**: 932-933 [PMID: 21568963]
13 **Barnes PM**, Powell-Griner E, McFann K, Nahin RL. Complementary and alternative medicine use among adults: United States, 2002. *Adv Data* 2004; 1-19 [PMID: 15188733]
14 **Pittler MH**, Karagülle MZ, Karagülle M, Ernst E. Spa therapy and balneotherapy for treating low back pain: meta-analysis of randomized trials. *Rheumatology* (Oxford) 2006; **45**: 880-884 [PMID: 16449365 DOI: 10.1093/rheumatology/kel018]
15 **Frumkin H**. Beyond toxicity: human health and the natural environment. *Am J Prev Med* 2001; **20**: 234-240 [PMID: 11275453]
16 **Ulrich RS**. Natural versus urban scenes: Some psychophysiological effects. *Environ Behav* 1981; **13**: 523-556 [DOI: 10.1177/0013916581135001]
17 **Morita E**, Fukuda S, Nagano J, Hamajima N, Yamamoto H, Iwai Y, Nakashima T, Ohira H, Shirakawa T. Psychological effects of forest environments on healthy adults: Shinrin-yoku (forest-air bathing, walking) as a possible method of stress reduction. *Public Health* 2007; **121**: 54-63 [PMID: 17055544 DOI: 10.1016/j.puhe.2006.05.024]
18 **Li Q**. Effect of forest bathing trips on human immune function. *Environ Health Prev Med* 2010; **15**: 9-17 [PMID: 19568839 DOI: 10.1007/s12199-008-0068-3]
19 World Health Organization. Available from: URL: http://www.who.int/topics/rehabilitation/en/. Accessed September 25, 2012
20 **Moher D**, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010; **340**: c869 [PMID: 20332511]
21 **Boutron I**, Moher D, Altman DG, Schulz KF, Ravaud P. Methods and processes of the CONSORT Group: example of an extension for trials assessing nonpharmacologic treatments. *Ann Intern Med* 2008; **148**: W60-W66 [PMID: 18283201]
22 **Hopewell S**, Clarke M, Moher D, Wager E, Middleton P, Alt-

man DG, Schulz KF. CONSORT for reporting randomised trials in journal and conference abstracts. *Lancet* 2008; **371**: 281-283 [PMID: 18221781 DOI: 10.1016/S0140-6736(07)61835-2]
23 **Hopewell S**, Ravaud P, Baron G, Boutron I. Effect of editors' implementation of CONSORT guidelines on the reporting of abstracts in high impact medical journals: interrupted time series analysis. *BMJ* 2012; **344**: e4178 [PMID: 22730543]
24 **Atkins D**, Best D, Briss PA, Eccles M, Falck-Ytter Y, Flottorp S, Guyatt GH, Harbour RT, Haugh MC, Henry D, Hill S, Jaeschke R, Leng G, Liberati A, Magrini N, Mason J, Middleton P, Mrukowicz J, O'Connell D, Oxman AD, Phillips B, Schünemann HJ, Edejer T, Varonen H, Vist GE, Williams JW, Zaza S. Grading quality of evidence and strength of recommendations. *BMJ* 2004; **328**: 1490 [PMID: 15205295 DOI: 10.1136/bmj.328.7454.1490]
25 International Prospective Register of Systematic Reviews. Available from: URL: http://www.crd.york.ac.uk/prospero/
26 **Booth A**, Clarke M, Ghersi D, Moher D, Petticrew M, Stewart L. An international registry of systematic-review protocols. *Lancet* 2011; **377**: 108-109 [PMID: 20630580 DOI: 10.1016/S0140-6736(10)60903-8]
27 **Szturm T**, Betker AL, Moussavi Z, Desai A, Goodman V. Effects of an interactive computer game exercise regimen on balance impairment in frail community-dwelling older adults: a randomized controlled trial. *Phys Ther* 2011; **91**: 1449-1462 [PMID: 21799138 DOI: doi: 10.2522/ptj.20090205]
28 **Gil-Gómez JA**, Lloréns R, Alcañiz M, Colomer C. Effectiveness of a Wii balance board-based system (eBaViR) for balance rehabilitation: a pilot randomized clinical trial in patients with acquired brain injury. *J Neuroeng Rehabil* 2011; **8**: 30 [PMID: 21600066 DOI: 10.1186/1743-0003-8-30]
29 **Saposnik G**, Teasell R, Mamdani M, Hall J, McIlroy W, Cheung D, Thorpe KE, Cohen LG, Bayley M. Effectiveness of virtual reality using Wii gaming technology in stroke rehabilitation: a pilot randomized clinical trial and proof of principle. *Stroke* 2010; **41**: 1477-1484 [PMID: 20508185 DOI: 10.1161/STROKEAHA.110.584979]
30 **Bauer CL**, Victorson D, Rosenbloom S, Barocas J, Silver RK. Alleviating distress during antepartum hospitalization: a randomized controlled trial of music and recreation therapy. *J Womens Health* (Larchmt) 2010; **19**: 523-531 [PMID: 20141383 DOI: 10.1089/jwh.2008.1344]
31 **Adamo KB**, Rutherford JA, Goldfield GS. Effects of interactive video game cycling on overweight and obese adolescent health. *Appl Physiol Nutr Metab* 2010; **35**: 805-815 [PMID: 21164552 DOI: 10.1139/H10-078]
32 **Hackney ME**, Earhart GM. Effects of dance on movement control in Parkinson's disease: a comparison of Argentine tango and American ballroom. *J Rehabil Med* 2009; **41**: 475-481 [PMID: 19479161 DOI: 10.2340/16501977-0362]
33 **Yavuzer G**, Senel A, Atay MB, Stam HJ. ''Playstation eyetoy games'' improve upper extremity-related motor functioning in subacute stroke: a randomized controlled clinical trial. *Eur J Phys Rehabil Med* 2008; **44**: 237-244 [PMID: 18469735]
34 **Desrosiers J**, Noreau L, Rochette A, Carbonneau H, Fontaine L, Viscogliosi C, Bravo G. Effect of a home leisure education program after stroke: a randomized controlled trial. *Arch Phys Med Rehabil* 2007; **88**: 1095-1100 [PMID: 17826452 DOI: 10.1016/j.apmr.2007.06.017]
35 **Higgins JPT**, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from: URL: http://www.cochrane.org/training/cochrane-handbook
36 **Sugiyama T**, Healy GN, Dunstan DW, Salmon J, Owen N. Joint associations of multiple leisure-time sedentary behaviours and physical activity with obesity in Australian adults. *Int J Behav Nutr Phys Act* 2008; **5**: 35 [PMID: 18590570 DOI: 10.1186/1479-5868-5-35]
37 **Dunstan DW**, Barr EL, Healy GN, Salmon J, Shaw JE, Balkau B, Magliano DJ, Cameron AJ, Zimmet PZ, Owen N. Television viewing time and mortality: the Australian Diabetes, Obesity and Lifestyle Study (AusDiab). *Circulation* 2010; **121**: 384-391 [PMID: 20065160 DOI: 10.1161/CIRCULATIONAHA.109.894824]
38 **Mark AE**, Janssen I. Relationship between screen time and metabolic syndrome in adolescents. *J Public Health* (Oxf) 2008; **30**: 153-160 [PMID: 18375469 DOI: 10.1093/pubmed/fdn022]
39 **Healy GN**, Dunstan DW, Salmon J, Shaw JE, Zimmet PZ, Owen N. Television time and continuous metabolic risk in physically active adults. *Med Sci Sports Exerc* 2008; **40**: 639-645 [PMID: 18317383 DOI: 10.1249/MSS.0b013e3181607421]
40 **Lyons EJ**, Tate DF, Ward DS, Bowling JM, Ribisl KM, Kalyararaman S. Energy expenditure and enjoyment during video game play: differences by game type. *Med Sci Sports Exerc* 2011; **43**: 1987-1993 [PMID: 21364477]
41 **Primack BA**, Carroll MV, McNamara M, Klem ML, King B, Rich M, Chan CW, Nayak S. Role of video games in improving health-related outcomes: a systematic review. *Am J Prev Med* 2012; **42**: 630-638 [PMID: 22608382 DOI: 10.1016/j.amepre.2012.02.023]
42 **Schwartz D**, Lellouch J. Explanatory and pragmatic attitudes in therapeutical trials. *J Clin Epidemiol* 2009; **62**: 499-505 [PMID: 19348976 DOI: 10.1016/j.jclinepi.2009.01.012]
43 **Bowen DJ**, Kreuter M, Spring B, Cofta-Woerpel L, Linnan L, Weiner D, Bakken S, Kaplan CP, Squiers L, Fabrizio C, Fernandez M. How we design feasibility studies. *Am J Prev Med* 2009; **36**: 452-457 [PMID: 19362699 DOI: 10.1016/j.amepre.2009.02.002]
44 **Gagnier JJ**, Boon H, Rochon P, Moher D, Barnes J, Bombardier C. Reporting randomized, controlled trials of herbal interventions: an elaborated CONSORT statement. *Ann Intern Med* 2006; **144**: 364-367 [PMID: 16520478 DOI: 10.7326/0003-4819-144-5-200603070-00013]
45 **Kamioka H**, Tsutani K, Okuizumi H, Mutoh Y, Ohta M, Handa S, Okada S, Kitayuguchi J, Kamada M, Shiozawa N, Honda T. Effectiveness of aquatic exercise and balneotherapy: a summary of systematic reviews based on randomized controlled trials of water immersion therapies. *J Epidemiol* 2010; **20**: 2-12 [PMID: 19881230 DOI: 10.2188/jea.JE20090030]
46 **Kamioka H**, Kuroyanagi R, Komatsu T, Kaminai T, Takahashi M, Mutoh Y, Tsutani K. A systematic review of randomized controlled trials on the therapeutic and health-promoting effects of spas. *J Jpn Assoc Balneol Climatol Phys Med* 2006; **69**: 155-166
47 **Liberati A**, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Ann Intern Med* 2009; **151**: W65-W94 [PMID: 19622512]
48 **Green J**, Forster A, Bogle S, Young J. Physiotherapy for patients with mobility problems more than 1 year after stroke: a randomised controlled trial. *Lancet* 2002; **359**: 199-203 [PMID: 11812553 DOI: 10.1016/S0140-6736(02)07443-3]
49 **Kobayashi R**, Ishigami K, Tukano M, Anezaki S, Nakadaira H. Effects of a fall prevention program on physical abilities of elderly people living in a rural region: an intervention trial. *Niigata Iryo Fukushi Gakkaishi* 2006; **5**: 18-26
50 **Das DA**, Grimmer KA, Sparnon AL, McRae SE, Thomas BH. The efficacy of playing a virtual reality game in modulating pain for children with acute burn injuries: a randomized controlled trial [ISRCTN87413556]. *BMC Pediatr* 2005; **5**: 1 [PMID: 15745448]
51 **Hurwitz EL**, Morgenstern H, Chiao C. Effects of recreational physical activity and back exercises on low back pain and psychological distress: findings from the UCLA Low Back Pain Study. *Am J Public Health* 2005; **95**: 1817-1824 [PMID: 16186460]

52 **Matsuo A**, Morioka S, Hiyamizu M, Maeoka H, Shomoto K. The influence of the exercise using a video game on the physical function and brain activities. *Physical Fitness Research Institute Research Aid Report* 2010; **25**: 82-90

53 **Saposnik G**, Mamdani M, Bayley M, Thorpe KE, Hall J, Cohen LG, Teasell R. Effectiveness of Virtual Reality Exercises in STroke Rehabilitation (EVREST): rationale, design, and protocol of a pilot randomized clinical trial assessing the Wii gaming system. *Int J Stroke* 2010; **5**: 47-51 [PMID: 20088994 DOI: 10.1111/j.1747-4949.2009.00404.x]

54 **Mitsumura M**, Someya F. A comparative study of ADL at home and at care facilities: differences between system of elderly daycare administration. *J Tsuruma Health Sci Soc Kanazawa Univ* 2011; **35**: 11-18

55 **Fraga MJ**, Cader SA, Ferreira MA, Giani TS, Dantas EH. Aerobic resistance, functional autonomy and quality of life (QoL) of elderly women impacted by a recreation and walking program. *Arch Gerontol Geriatr* 2011; **52**: e40-e43 [PMID: 20554333 DOI: 10.1016/j.archger.2010.04.021]

56 **Watanabe Y**. Effects of congnitive rehabilitation with computer training on neurophychological function in schizophrenia. *Seishin Igaku* 2011; **53**: 865-874

57 **Hsu JK**, Thibodeau R, Wong SJ, Zukiwsky D, Cecile S, Walton DM. A "Wii" bit of fun: the effects of adding Nintendo Wii(®) Bowling to a standard exercise regimen for residents of long-term care with upper extremity dysfunction. *Physiother Theory Pract* 2011; **27**: 185-193 [PMID: 20698793 DOI: 10.3109/09593985]

58 **Kwok BC**, Mamun K, Chandran M, Wong CH. Evaluation of the Frails' Fall Efficacy by Comparing Treatments (EFFECT) on reducing fall and fear of fall in moderately frail older adults: study protocol for a randomised control trial. *Trials* 2011; **12**: 155 [PMID: 21682909 DOI: 10.1186/1745-6215-12-155]

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Periodontal disease is associated with increased coronary heart disease risk: A meta-analysis based on 38 case-control studies

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Abstract

AIM: To investigate whether periodontal disease (PD) is associated with increasing coronary heart disease (CHD) risk by performing a meta-analysis.

METHODS: Two authors independently searched PubMed and China National Knowledge Infrastructure up to January 10th, 2013 for relevant case-control studies that investigated the association between PD and CHD. After quality assessment using Newcastle-Ottawa Scale and data extraction by two independent authors, the overall and subgroup meta-analyses were performed and publication bias were examined using the Comprehensive Meta-Analysis V2 software. Potential publication bias was assessed using visual inspection of the funnel plots, Egger linear regression test, and trims and fill method.

RESULTS: Finally 38 relevant case-control studies were identified, involving 4950 CHD patients and 5490 controls. Eleven studies were rated low quality and 27 were high quality. Based on random-effects, a significant association was identified between PD and CHD (OR 3.79, 95%CI: 2.23-6.43, $P < 0.001$, $I^2 = 98.59\%$), and sensitivity analysis showed that this result was robust. Subgroup analyses according to adjusted/unadjusted ORs, source of control, methodological quality, end point, assessment of PD/CHD, and ethnicity also indicated a significant association. Publication bias was detected, and the estimated OR including the "missing" studies did not substantially differ from our estimate with adjustment for missing studies (OR 4.15, 95%CI: 2.62-6.54, $P < 0.001$).

CONCLUSION: Based on the meta-analysis, PD is probably associated with CHD risk independently and significantly.

Key words: Periodontal disease; Coronary heart disease; Case-control study; Risk factor; Meta-analysis

Core tip: Growing evidence indicated that periodontal disease (PD) might be associated with coronary heart disease (CHD), however, results from the studies were inconsistent. This meta-analysis based on 38 case-control studies indicated that PD increased a 3.79-fold risk of CHD (OR = 3.79, 95%CI: 2.23-6.43, $P < 0.001$, $I^2 = 98.59\%$). The results showed that PD is probably an independent and significant risk factor for CHD.

Leng WD, Zeng XT, Chen YJ, Zhan ZQ, Yang Y. Periodontal disease is associated with increased coronary heart disease risk: A meta-analysis based on 38 case-control studies. *World J Meta-Anal* 2013; 1(1): 47-56 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i1/47.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i1.47

INTRODUCTION

Coronary heart disease (CHD) is one of the major causes of mortality, account for nearly 30% of deaths worldwide[1]. As almost half of all first onset of CHD events occur in asymptomatic patients[2], it is important to seek CHD risk factors and accurately identify high-risk individuals and guide the risk reduction interventions, prevention, and lifestyle changes. CHD is a complex disease, epidemiologic studies have suggested that the etiology of CHD involved interactions of genetics, environmental factors, and gene-gene and gene-environment[3]. Environmental factors (including psychological and social factors)[4,5] are the classical risk factors for CHD, however, these markers do not explain the etiology of CHD to the fullest of its extent. Given the importance of fatal health problem related to CHD, efforts are being made to identify other modifiable risk factors that play a role in the etiology of CHD.

Periodontal disease (PD) is a group of inflammatory diseases which affect the supporting tissues of the tooth, approximately at least 35% dentate adults aged 30-90 years in United States suffer from PD[6], and it also affects up to 90% of the worldwide population[7]. Based on the theory of "focal infection" which emerged at the beginning of the 20th century, many studies have observed a possible role of PD as a risk factor for systemic conditions over the past two decades[8], such as cardiovascular diseases[9], diabetes[10], and chronic obstructive pulmonary disease[11].

Growing evidences indicated that chronic infections and inflammation (such as PD) might play a role in the initiation and progression of CHD[12]. Many epidemiological studies have investigated the link between PD and risk of CHD, and most of them found a positive association, even though some results are varied or even contradictory among studies. There was a published meta-analysis based on 8 cross sectional and 14 case-control studies by Blaizot *et al*[9] in 2009, which identified that there were higher odds of developing CHD in patients with PD (OR 2.35, 95%CI: 1.87-2.96, $P < 0.0001$). However, this meta-analysis did not perform subgroup analyses because of the study design, and adjusted or unadjusted factors. As we know, a cross sectional design is subjected to more confounding and biases than a case-control design, and adjusted data could obtain more precise point estimate than unadjusted data. Up to now, there have been 38 case-control studies[13-50] published in English or Chinese.

An improved understanding of this association may have important public health and clinical implications, for prevention and treatment of PD would reduce the CHD events. This meta-analysis aims to (1) evaluate the inconsistent results from published case-control studies on the association between PD and risk of CHD; (2) gain a more precise estimate association; and (3) provide a general interpretation of the results in the context of other evidences and propose suggestions for the prevention and treatment of the diseases.

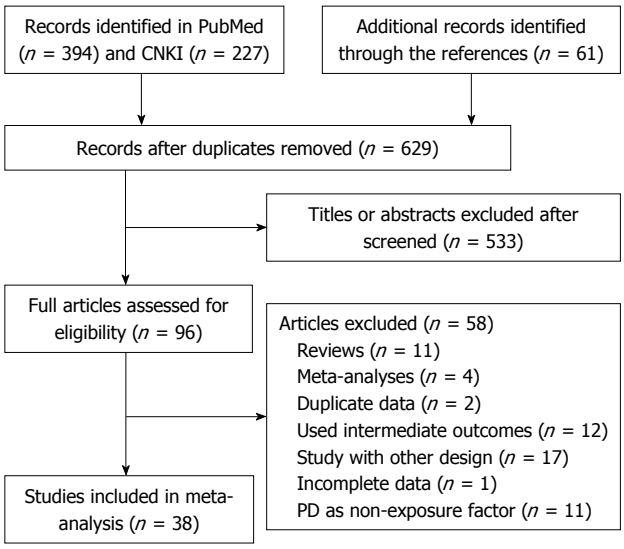

Figure 1 Flow chart of included case-control studies that tested the association between periodontal disease and risk of coronary heart disease. PD: Periodontal disease; CHD: Coronary heart disease; CNKI: China National Knowledge Infrastructure.

MATERIALS AND METHODS

We followed the proposed MOOSE (Meta-Analysis of Observational Studies in Epidemiology)[51] guidelines to report the present meta-analysis.

Literature search

We initially identified published studies that investigated the association between PD and CHD by searching the PubMed and China National Knowledge Infrastructure databases up to January 10th, 2013. The following search terms were used: (1) "PD" or "peridontal disease" or "periodontitis" or "periodontal attachment loss" or "periodontal pocket" or "alveolar bone loss", and (2) "CHD" or "coronary artery disease" or "myocardial infarction (MI)" or "angina pectoris" or "ischemic heart disease". The studies were published in either English or Chinese. We also reviewed the reference lists of retrieved articles, previous meta-analysis, and recent reviews.

Study selection

Any study met all of the following criteria was included: (1) the study was of a case-control design; (2) clear diagnostic criteria for PD and CHD were reported; (3) the association between PD and risk of CHD was investigated, and PD is the exposed factor; and (4) the ORs and the

Table 1 Characteristics and methodological quality of included 38 case-control studies in the meta-analysis

Ref.	Location	Sample size	Age (case/control, yr)	Source of control	Assessment		End points	OR (95%CI)	NOS
					PD	CHD			
Li *et al*[13]	China	88/128	> 60	Hospital-based	PI	C	CHD	1.85 (1.07-3.20)	4
López *et al*[14]	Chile	35/51	42.5 ± 5.7/40.5 ± 6.3	Hospital-based	PPD	ECG	CHD	3.17 (1.31-7.65)[1]	6
Huang *et al*[15]	China	146/136	58.7 ± 8.9	Hospital-based	Q	CAG	CHD	2.27 (1.40-3.68)	5
Liu *et al*[16]	China	216/216	59.4 ± 15.3/57.9 ± 13.7	Population-based	PI	CAG	CHD	5.42 (3.32-8.86)	4
Rutger Persson *et al*[17]	Sweden	80/80	63.4 ± 8.9/61.9 ± 9.1	Population-based	ABL	ECG	MI	14.1 (5.8-34.4)[1]	7
Geerts *et al*[18]	Belgium	108/62	59.2 ± 10.9/57.7 ± 8.7	Hospital-based	PPD	C	CHD	6.50 (1.80-23)[1]	7
Montebugnoli *et al*[19]	Italy	63/50	52.3 ± 4.9/54.5 ± 6.1	Population-based	PPD	CAG	CHD	4.61 (1.00-23.20)[1]	8
Renvert *et al*[20]	Sweden	88/80	62.7 ± 9.1/NA	Hospital-based	PPD	C	MI	7.67 (1.13-51.92)[1]	6
Tang *et al*[21]	China	250/250	⩾ 45	Hospital-based	CPI	C	CHD	1.95 (1.36-2.78)	4
Buhlin *et al*[22]	Sweden	143/50	65.9 ± 8.6/64.5 ± 8.3	Population-based	PPD	CAG	CHD	3.80 (1.68-8.74)[1]	8
Liu *et al*[23]	China	45/40	54.9 ± 8.1/51.2 ± 6.5	Hospital-based	PI	CAG	CHD	18.70 (6.25-55.93)	4
Wang *et al*[24]	China	216/216	59 ± 15/58 ± 14	Population-based	ABL	CAG	CHD	1.76 (1.31-2.36)[1]	7
Andriankaja *et al*[25]	United States	537/800	54.6 ± 8.5/55.0 ± 0.0	Population-based	CAL	C	MI	2.24 (1.60-3.13)[1]	8
Barilli *et al*[26]	Brazil	40/59	49.2 (30-79)	Hospital-based	CPI	C	CHD	61 (17.26-214.86)	5
Briggs *et al*[27]	United Kingdom	92/79	56.7 ± 6.3/58.2 ± 6.7	Population-based	PPD	CAG	CHD	3.06 (1.02-9.17)[1]	8
Geismar *et al*[28]	Denmark	110/140	65/62.6	Hospital-based	ABL	ECG	CHD	2.0 (0.77-5.08)[1]	7
Li *et al*[29]	China	357/305	72.5 ± 8.9	Population-based	PI	CAG	CHD	1.16 (0.91-1.48)	6
Spahr *et al*[30]	Germany	263/526	61.0 ± 7.1/61.0 ± 7.1	Population-based	CPI	CAG	CHD	1.67 (1.08-2.58)[1]	8
Zhang *et al*[31]	China	77/74	50.2 ± 9.6/50.8 ± 9.5	Population-based	PPD	CAG	CHD	2.13 (1.08-4.22)	6
Zhang *et al*[32]	China	277/238	57 ± 11.3/55 ± 10.8	Hospital-based	CAL	CAG	CHD	2.70 (1.52-4.80)[1]	7
Latronico *et al*[33]	Italy	15/19	57.7/55.1	Population-based	ABL	CAG	CHD	5.85 (1.03-33.12)	6
Nonnenmacher *et al*[34]	Germany	45/45	63.5 ± 7.4/63.6 ± 7.4	Hospital-based	CAL	CAG	CHD	3.2 (1.2-9.0)[1]	7
Rech *et al*[35]	Brazil	58/57	59.3/70	Hospital-based	PPD	ECG	MI	1.8 (0.7-4.7)[1]	6
Ge *et al*[36]	China	13/30	55.1 ± 4.8/51.2 ± 4.7	Hospital-based	CAL	CAG	CHD	2.53 (1.01-6.32)[1]	6
Meng *et al*[37]	China	150/150	71.2 ± 4.6/71.9 ± 4.7	Population-based	ABL	C	CHD	2.95 (1.74-5.02)	5
Wu *et al*[38]	China	77/75	53.81 ± 8.25/ 51.14 ± 6.44	Hospital-based	CAL	CAG	CHD	2.18 (1.52-3.13)	5
Zamirian *et al*[39]	Iran	80/80	54.0 ± 8.7/51.9 ± 9.4	Hospital-based	CAL	ECG	MI	3.18 (1.37-7.42)[1]	6
Zhu *et al*[40]	China	98/104	61.34 ± 9.63	Population-based	CAL	C	MI	11.43 (2.59-50.34)	5
Dong *et al*[41]	China	161/162	33-66/30-70	Population-based	ABL	CAG	CHD	5.74 (2.07-15.90)[1]	7
Ma *et al*[42]	China	146/257	45-72	Hospital-based	CAL	CAG	CHD	2.36 (1.49-3.73)	5
Oikarinen *et al*[43]	Kuwait	88/88	48.8 ± 10.0/47.0 ± 11.6	Hospital-based	ABL	C	CHD	19.69 (19.36-20.02)	6
Sun *et al*[44]	China	167/242	68.28 ± 10.53/ 50.18 ± 10.56	Hospital-based	CAL	CAG	CHD	9.10 (0.87-95.07)[1]	7
Willershausen *et al*[45]	Germany	125/125	61.8 ± 10.4/63.4 ± 10.7	Population-based	CAL	ECG	MI	3.65 (2.02-6.56)[1]	7
Bokhari *et al*[46]	Pakistan	45/35	41.67 ± 5.11/ 40.31 ± 6.97	Hospital-based	PPD	CAG	CHD	6.37 (1.26-32.27)	6
Chen[47]	China	46/34	38-68/35-66	Hospital-based	ABL	CAG	CHD	9.87 (3.50-27.82)	4
Sikka *et al*[48]	India	100/100	54.97 ± 7.97/ 55.1 ± 8.08	Population-based	CPI	CAG	CHD	2.66 (1.50-4.71)	7
Ashraf *et al*[49]	Pakistan	145/145	53.3 ± 12.3/51.7 ± 11.6	Hospital-based	CPI	C	CHD	1.20 (0.93-1.55)[1]	7
Zhang *et al*[50]	China	162/162	66.7/66.0	Hospital-based	Q	CAG	CHD	2.16 (1.65-2.83)[1]	6

[1]Adjusted OR and 95%CI. PD: Periodontal disease; CHD: Coronary heart disease; NOS: Newcastle-Ottawa Scale; CAG: Coronary arteriography; ECG: Electrocardiograph; MI: Myocardial infarction; CAL: Clinical attachment loss; PPD: Periodontal pocket depth; ABL: Alveolar bone loss; CPI: Community periodontal index; PI: Periodontal index; Q: Questionnaire; C: Cardiologist.

corresponding 95%CIs, or the number of events were reported. Two authors independently evaluated the eligibility of all retrieved studies; disagreements were resolved by discussion or consultation with a third author.

Methodological quality assessment

The methodological quality of included studies was assessed independently by two authors according to the Newcastle-Ottawa Scale (NOS) for case-control study[52]. The NOS for case-control study consists of 3 parameters of quality: selection, comparability, and exposure assessment. It assigns a maximum of 4 points for selection, a maximum of 2 points for comparability, and a maximum of 3 points for exposure. Therefore, 9 points is the highest score, reflecting the highest quality. We defined overall quality rating scores < 6 as low quality, and ⩾ 6 as high quality. All discrepancies between authors were addressed by a common reevaluation of the original article.

Data extraction

Two authors independently extracted data of each study using a preliminary standardized data collection form. Data extracted included: first author's last name, year of publication, country of study; characteristics of study

Table 2 Adjustments in case-control studies included in this meta-analysis

Ref.	Adjustment
López *et al*[14]	DM, systolic blood pressure, and smoking
Rutger Persson *et al*[17]	Smoking
Geerts *et al*[18]	Age, gender, smoking, DM, hypertension, hyperlipidemia, diet, and alcohol
Montebugnoli *et al*[19]	Age, smoking, DM, hypertension, high/low density lipoprotein, CRP, leukocytes, BMI, social class
Renvert *et al*[20]	Smoking
Buhlin *et al*[22]	Age, gender, smoking, DM, BMI, education, place of birth
Wang *et al*[24]	Gender, age, BMI, smoking, hypertension, DM, blood lipid, CRP, white blood count, and fibrinogen
Andriankaja *et al*[25]	Age, gender, hypertension, cholesterol, DM, and smoking
Briggs *et al*[27]	Smoking, education, alcohol, BMI, exercise, unemployment, hobby, plaque, and CRP
Geismar *et al*[28]	Gender, smoking, DM, and education
Spahr *et al*[30]	Age, sex, BMI, smoking, alcohol, DM, hypertension, hyperlipoproteinemia, education, exercise, and statin intake
Zhang *et al*[32]	Smoking, age, gender, BMI, hypertension, DM, high-density lipoproteincholesterol, total Cholesterol, total glycerin
Nonnenmacher *et al*[34]	Smoking and BMI
Rech *et al*[35]	Age, gender, smoking, DM
Ge *et al*[36]	Blood pressure and BMI
Zamirian *et al*[39]	Smoking and alcohol
Dong *et al*[41]	Smoking, age, and education
Sun *et al*[44]	Age and BMI
Willershausen *et al*[45]	Age, gender, and smoking
Ashraf *et al*[49]	Age, gender, and education
Zhang *et al*[50]	Age, gender, smoking, alcohol, hypertension, and BMI

DM: Diabetes mellitus; BMI: Body mass index; CRP: C-reactive protein.

population and age at baseline; number of participants with PD and CHD, and total number of participants, or ORs and relevant 95%CIs; end points of CHD, ascertainment of PD and CHD; and adjustment for covariates. Any disagreement was resolved by consensus. CHD was defined as MI, angina pectoris, and other ischemic heart diseases (IHD).

Statistical analysis

We pooled the results from single studies which were found to be both clinically and statistically appropriate. We computed pooled ORs and relevant 95%CIs using Comprehensive Meta-Analysis software, Version 2.2 (Biostat, Englewood, NJ, United States)[53], to generate forest plots, determine whether a statistical association between PD and CHD exists, assess the heterogeneity of the selected studies, and detect whether publication bias present. Heterogeneity was quantified using the I^2 statistic[54], with the low, moderate, and high I^2 values of 25%, 50%, and 75%, respectively[55], where I^2 value of 25% or lower indicated no evidence of heterogeneity, we used the fixed-effect model; otherwise, the random-effects model was used.

When heterogeneity existed, we performed subgroup and sensitivity analyses to explore possible explanations for the heterogeneity and examine the influence of various exclusion criteria on the overall risk estimate. We also investigated the influence of single study on the overall risk estimate by sequentially removing each study to test the robustness of the main results.

Potential publication bias was assessed by visual inspection of the funnel plots of overall outcome. The Egger linear regression test was used to examine the association between mean effect estimate and its variance[56]. In addition, to assess the effect of possible publication bias, we calculated the number of unpublished studies which may exist to negate the results, and the pooled OR adjusted for publication bias using the trim and fill method[57].

RESULTS

Study identification

Of 682 records searched initially, 38 case-control studies[13-50] were included in this meta-analysis. A detailed flowchart of the selection process is shown in Figure 1.

Characteristics and quality of studies

Table 1 presents the major characteristics and methodological quality of the 38 case-control studies. These studies focused on CHD only. Sample sizes ranged from 34 to 1337, involving 4950 CHD patients and 5490 controls subjects. Twenty-one studies[14,17-20,22,24,25,27,28,30,32,34-36,39,41,44,45,49,50] were adjusted covariates (Table 2), while there was no adjustment of the other 17 studies[13,15,16,21,23,26,29,31,33,37,38,40,42,43,46-48]. The methodological quality of 11 studies[13,15,16,21,23,26,37,38,40,42,47] according to NOS were rated low quality, and 27 were rated high quality[14,17-20,22,24,25,27-36,39,41,43-46,48-50]. All the CHD patients were confirmed and non-CHD patients were excluded by coronary arteriography (CAG), cardiologists, or electrocardiography (ECG).

PD and risk of CHD

Of all 38 studies, six studies[19,28,29,35,44,49] showed no statistical difference, and all the 38 studies identified significantly increased risk of developing CHD (OR 3.79, 95%CI: 2.23-6.43, $P < 0.001$). Substantial heterogeneity was observed ($I^2 = 98.59\%$, $P < 0.001$). Figure 2 shows the results from the random-effects model pooling the ORs and 95%CIs.

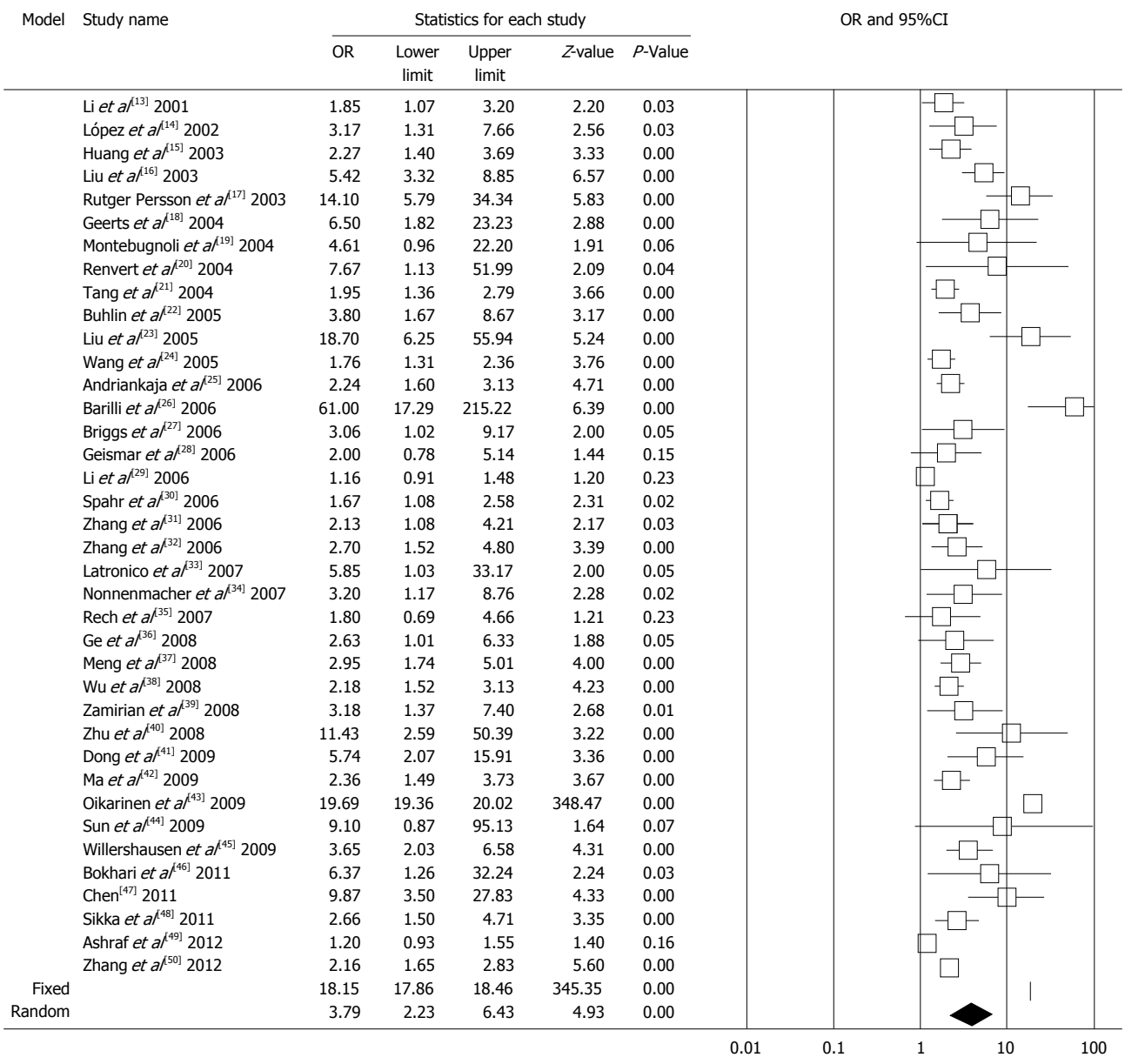

Figure 2 Forest plot of periodontal disease and risk of coronary heart disease, using pooled random-effects model. The pooled odds ratio is represented by a diamond of standard height, with the width indicating the 95%CI.

Subgroup and sensitivity analyses

Table 3 shows the results of subgroup analyses by adjustment for covariates, source of control, methodological quality, end point, assessment of PD, assessment of CHD, and ethnicity. All these analyses indicated that PD is a risk factor for CHD. Sensitivity analysis was performed by sequentially removing each study, the significance of pooled ORs was not influenced by the omission of any single study (the values of ORs were between 3.05 and 3, 91, and the relevant 95%CIs between 2.06 and 6.62), suggesting that the results of this meta-analysis were stable (Figure 3).

Publication bias

Figure 4 shows that the funnel plot was asymmetrical, indicating publication bias existed; this was also confirmed by Egger linear regression test ($P < 0.001$). As the evidence of bias could be due to inadequate statistical power, we used the non-parametric method of "trim and fill" and estimated 3 possible missing studies based on random-effects model (black spots in Figure 4). The estimated OR including the "missing" studies did not substantially differ from our estimate with adjustment for missing studies (OR 4.15, 95%CI: 2.62-6.54, $P < 0.001$).

DISCUSSION

In 1989, Mattila *et al*[58] reported that dental health was significantly associated with acute MI, and this association remained valid after adjustment for age, social class, smoking, serum lipid concentrations, and the presence of diabetes. Since then, many observational studies have emerged to investigate the relationship between

Study name	Statistics with study removed				
	Point	Lower limit	Upper limit	*Z*-value	*P*-value
Li *et al*[13] 2001	3.86	2.26	6.61	4.93	0.00
López *et al*[14] 2002	3.80	2.22	6.51	4.87	0.00
Huang *et al*[15] 2003	3.84	2.24	6.58	4.90	0.00
Liu *et al*[16] 2003	3.75	2.18	6.45	4.77	0.00
Rutger Persson *et al*[17] 2003	3.65	2.13	6.26	4.71	0.00
Geerts *et al*[18] 2004	3.74	2.18	6.39	4.81	0.00
Montebugnoli *et al*[19] 2004	3.77	2.20	6.44	4.85	0.00
Renvert *et al*[20] 2004	3.73	2.18	4.37	4.82	0.00
Tang *et al*[21] 2004	3.86	2.26	6.60	4.93	0.00
Buhlin *et al*[22] 2005	3.79	2.21	6.40	4.85	0.00
Liu *et al*[23] 2005	3.63	2.12	6.22	4.70	0.00
Wang *et al*[24] 2005	3.87	2.27	6.61	4.96	0.00
Andriankaja *et al*[25] 2006	3.85	2.24	6.59	4.90	0.00
Barilli *et al*[26] 2006	3.53	2.06	6.04	4.60	0.00
Briggs *et al*[27] 2006	3.61	2.23	6.52	4.88	0.00
Geismar *et al*[28] 2006	3.85	2.25	6.59	4.92	0.00
Li *et al*[29] 2006	3.91	2.33	6.56	5.16	0.00
Spahr *et al*[30] 2006	3.88	2.27	6.62	4.96	0.00
Zhang *et al*[31] 2006	3.85	2.25	6.59	4.91	0.00
Zhang *et al*[32] 2006	3.82	2.23	6.55	4.88	0.00
Latronico *et al*[33] 2007	3.75	2.19	6.41	4.83	0.00
Nonnenmacher *et al*[34] 2007	3.80	2.22	6.51	4.87	0.00
Rech *et al*[35] 2007	3.86	2.26	6.61	4.94	0.00
Ge *et al*[36] 2008	3.83	2.24	6.55	4.90	0.00
Meng *et al*[37] 2008	3.81	2.22	6.54	4.86	0.00
Wu *et al*[38] 2008	3.85	2.25	6.59	4.90	0.00
Zamirian *et al*[39] 2008	3.80	2.22	6.52	4.87	0.00
Zhu *et al*[40] 2008	3.69	2.16	6.31	4.76	0.00
Dong *et al*[41] 2009	3.74	2.19	6.41	4.81	0.00
Ma *et al*[42] 2009	3.84	2.24	6.58	4.89	0.00
Oikarinen *et al*[43] 2009	3.05	2.48	3.75	10.52	0.00
Sun *et al*[44] 2009	3.72	2.18	6.36	4.82	0.00
Willershausen *et al*[45] 2009	3.79	2.21	6.50	4.84	0.00
Bokhari *et al*[46] 2011	3.74	2.19	6.40	4.82	0.00
Chen[47] 2011	3.69	2.15	6.32	4.76	0.00
Sikka *et al*[48] 2011	3.82	2.23	6.55	4.88	0.00
Ashraf *et al*[49] 2012	3.91	2.32	6.58	5.12	0.00
Zhang *et al*[50] 2012	3.85	2.24	6.60	4.90	0.00
	3.79	2.23	6.43	4.93	0.00

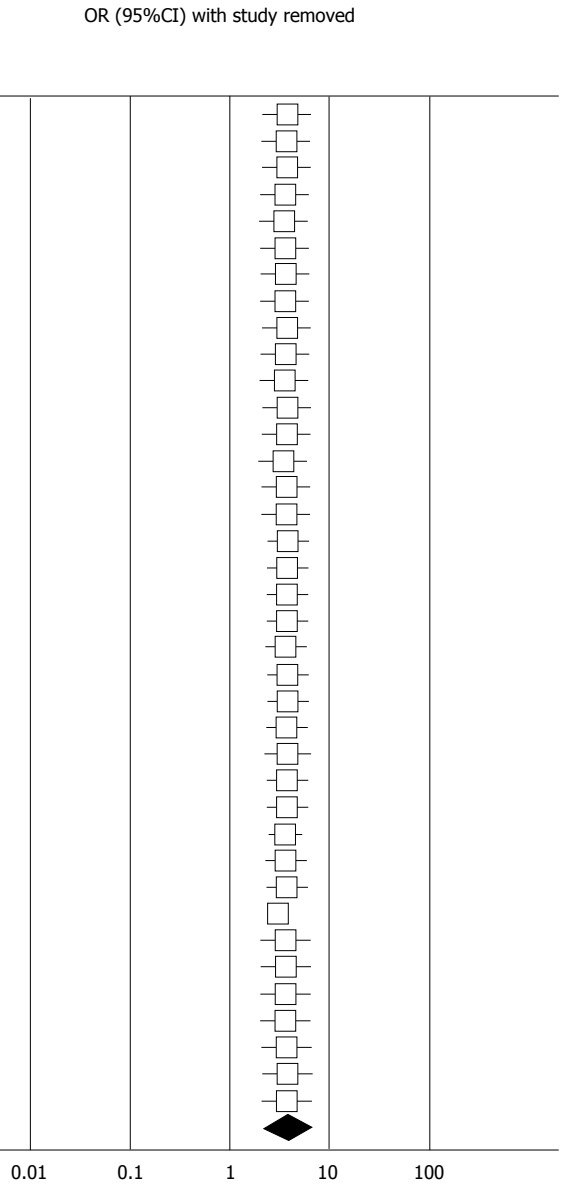

Figure 3 Forest plot of sensitivity analysis by removing each study in each turn. The pooled odds ratio is represented by a diamond of standard height, with the width indicating the 95%CI.

oral health and CHD, and PD was of special concern. However, the result remains controversial. In the present study, we performed a meta-analysis about the association between PD and CHD risk based on 38 case-control studies, and identified that subjects with PD had higher odds and higher risk of developing CHD than subjects without PD. Subgroup analyses based on adjustment for covariates, source of control, methodological quality, end point, assessment of PD, assessment of CHD, and ethnicity yielded significant and consistent results.

Compared with the previous meta-analysis by Blaizot *et al*[9] in 2009, whose result based on pooled 8 cross-sectional and 14 case-control studies identified 2.35 times higher risk of developing CHD in patients with PD (OR 2.35, 95%CI: 1.87-2.96, $P < 0.001$), our meta-analysis identified the higher risk (OR 3.79, 95%CI: 2.23-6.43, $P < 0.001$) based on 38 case-control studies. Obviously, our meta-analysis separated case-control studies from cross-sectional studies, therefore, the result was subjected to fewer confounding and biases of study design.

Second, except for geographic area, end point, and assessment of PD, we added subgroup analysis according to adjustment for covariates, source of control, methodological quality, and assessment of CHD. We found that the populations of America (OR 4.75, 95%CI: 1.50-15.02) had higher risk than Europeans (OR 3.81, 95%CI: 2.46-5.91), and Europeans had higher risk than Asians (OR 3.46, 95%CI: 1.73-6.94). This was different from previous meta-analysis, whose result showed American populations seem to present weaker association between PD and CHD than European ones. If it is due to the individual and social economic factors, why the oral health awareness and healthcare level of European and American populations are higher than Asians, but the trend of

Table 3 Results of overall and subgroups analyses of pooled odds ratios and 95%CIs

Total and subgroup	No. of trials	Heterogeneity		Model	Meta-analysis		
		I^2 (%)	*P*		OR	95%CI	*P*
Total	38	98.59	< 0.001	Random	3.79	2.23-6.43	< 0.001
Adjustment for covariates							
Yes	21	66.02	< 0.001	Random	2.72	2.13-3.46	< 0.001
No	17	98.68	< 0.001	Random	4.62	2.10-10.18	< 0.001
Source of control							
HB	22	98.5	< 0.001	Random	4.04	2.00-8.12	< 0.001
PB	16	80.38	< 0.001	Random	3.08	2.23-4.26	< 0.001
Methodological quality (NOS)							
< 6	11	83.28	< 0.001	Random	3.38	1.75-6.52	< 0.001
⩾ 6	27	98.73	< 0.001	Random	4.16	2.71-6.40	< 0.001
End point							
CHD	31	98.75	< 0.001	Random	3.63	2.00-6.59	< 0.001
MI	7	70.15	< 0.001	Random	4.12	2.35-7.22	< 0.001
Assessment of PD							
ABL	8	97.93	< 0.001	Random	5.57	1.90-16.33	< 0.001
CAL	10	0	0.5	Fixed	2.54	2.13-3.04	< 0.001
CPI	5	90.24	< 0.001	Random	2.76	1.45-5.23	< 0.001
PI	5	91.95	< 0.001	Random	3.12	1.38-7.05	< 0.001
PPD	8	0	0.76	Fixed	3.55	2.39-5.27	< 0.001
Questionnaire	2	0	0.86	Fixed	2.19	1.73-2.77	< 0.001
Assessment of CHD							
CAG	22	73.04	< 0.001	Random	2.85	2.23-3.64	< 0.001
Cardiologists	10	99	< 0.001	Random	5.09	1.71-15.14	< 0.001
ECG	6	60.72	0.03	Random	3.55	2.06-6.12	< 0.001
Ethnicity							
America	4	88.24	< 0.001	Random	4.75	1.50-15.02	0.01
Asia	23	99.01	< 0.001	Random	3.46	1.73-6.94	< 0.001
Europe	11	56.82	0.01	Random	3.81	2.46-5.91	< 0.001

PD: Periodontal disease; CHD: Coronary heart disease; NOS: Newcastle-Ottawa Scale; OR: Odds ratio; CAG: Coronary arteriography; ECG: Electrocardiograph; MI: Myocardial infarction; CAL: Clinical attachment loss; PPD: Periodontal pocket depth; ABL: Alveolar bone loss; CPI: Community periodontal index; PI: Periodontal index.

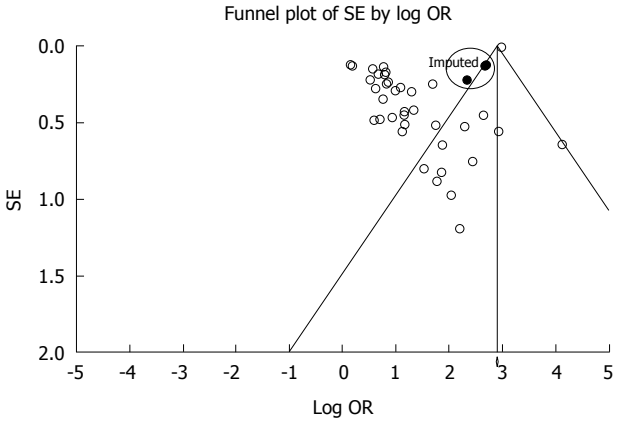

Figure 4 Filled funnel plot with pseudo-95%CIs of the 38 studies. Log of odds ratio (OR) represents the natural logarithm of the OR of individual studies; Standard error by Log OR represents the standard error in the natural logarithm of the OR of individual studies. A circle in the figure represents a study, while a black spot represents an unpublished study which may exist to negate the results of the meta-analysis.

risk is just opposite. Whether other factors, such as racial predisposition of CHD or dietary difference (*e.g.*, Asians like drinking green tea) caused this result still needs to be identified by further researches.

Third, when stratified by adjustment for covariates, the risk of adjusted data was lower (OR 2.72, 95%CI: 2.13-3.46, $P < 0.001$) than unadjusted ones (OR 4.62, 95%CI: 2.10-10.18, $P < 0.001$), and the relevant 95%CI was also narrower. This showed that adjusted data could obtain more precise point estimate than unadjusted data, and also confirmed that PD was an independent risk factor of CHD. When stratified by assessment of CHD, we observed that a definite diagnosis by cardiologists showed higher risk (OR 5.09, 95%CI: 1.71-15.14) than by CAG (OR 2.85, 95%CI: 2.23-3.64) or ECG (OR 2.06, 95%CI: 2.06-6.12). This may be because that objective diagnostic approach is more accurate than subjective one, therefore, similar researches in future combining objective and subjective diagnostic approaches would be more beneficial to confirm CHD. The high methodological quality studies obtained narrower CI (OR 4.16, 95%CI: 2.71-6.40) than low quality ones (OR 3.38, 95%CI: 1.75-6.52), and this was in accordance with PB (OR 3.08, 95%CI: 2.23-4.26) compared with HB (OR 4.04, 95%CI: 2.00-8.12). It could be concluded that high methodological quality and PB study can more effectively control confounding bias.

Some limitations also should be indicated. The major limitation of this meta-analysis was the clinical heterogeneity among the studies with regard to both outcome and exposure definitions. Although we performed subgroup analyses according to the possible sources of heteroge-

neity, clinical heterogeneity also could not be removed completely. However, the result of sensitivity analysis supported that overall result was not influenced by any included single study. Second, 17 studies did not adjust covariates, and the pooled results also showed that the risk reduction sequence was unadjusted, followed by total (combined with adjusted and unadjusted), and adjusted. This means that the overall result may exaggerate the risk. Third, this study only included articles published in Chinese and English, and articles in the other languages (representing the populations of other races) were underrepresented. The funnel plot, Egger linear regression test, and "trim and fill" method also indicated publication bias. Finally, according to the the American Association of Periodontology in 1999, PD should be confirmed by the measure of clinical attachment loss (CAL). However, other periodontal outcomes such as periodontal pocket depth, alveolar bone loss, community periodontal index, periodontal index, or by dentist according to questionnaire were conducted in 28 included studies. This variety of criteria leads to careful interpretation of the meta-analysis results.

In conclusion, this meta-analysis indicated that PD was associated with CHD risk independently and significantly, and we can conclude that an effective oral hygiene regimen would effectively prevent the progression of CHD, an effective PD intervention treatment can control CHD, and correct and effective brushing of teeth, use of dental floss, and regular periodontal scaling would be the simplest and most cost-effective actions. However, whether this is a causal association or PD is only a marker of CHD needs to be confirmed by well-designed studies with larger sample sizes and by taking the certain genetic or environmental confounding factors into account; and whether periodontal interventions are effective also needs to be validated by high quality studies with strict design, large sample size and the standardized implementation, and multi-center randomized controlled trials.

COMMENTS

Background

Growing evidence indicated that periodontal disease (PD) might be associated with coronary heart disease (CHD), however, results from these studies were inconsistent. Thus, whether PD is a risk factor of CHD remains to be clarified.

Research frontiers

CHD is one of the major causes of mortality, account for nearly 30% of deaths worldwide; PD is one of the major two oral diseases and affect up to 90% of the worldwide population. Therefore, it is very important to identify the association between PD and CHD, in order to provide evidence for prevention and treatment of the diseases.

Innovations and breakthroughs

This is a comprehensive meta-analysis, in which the authors performed subgroup analyses to identify the similarities and differences between the adjustment for covariates, source of control, methodological quality, end point, assessment of PD, assessment of CHD, and ethnicity. All these analyses indicated that PD is a risk factor for CHD.

Applications

According to this meta-analysis, an effective oral hygiene regimen would effectively prevent the progression of CHD, an effective PD intervention treatment can control CHD, and correct and effective brushing of teeth, use of dental floss, and regular periodontal scaling would be the simplest and most cost-effective actions. In addition, whether this is a causal association or PD is only a marker of CHD, and whether periodontal interventions are effective remain to be confirmed.

Peer review

The authors have made a good meta-analysis, complete and deep enough.

REFERENCES

1 **Negi S**, Anand A. Atherosclerotic coronary heart disease-epidemiology, classification and management. *Cardiovasc Hematol Disord Drug Targets* 2010; **10**: 257-261 [PMID: 20932265 DOI: 10.2174/187152910793743832]

2 **Rosamond W**, Flegal K, Friday G, Furie K, Go A, Greenlund K, Haase N, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell CJ, Roger V, Rumsfeld J, Sorlie P, Steinberger J, Thom T, Wasserthiel-Smoller S, Hong Y. Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2007; **115**: e69-171 [PMID: 17194875 DOI: 10.1161/CIRCULATIONAHA.106.179918]

3 **Mi X**, Eskridge KM, George V, Wang D. Structural equation modeling of gene-environment interactions in coronary heart disease. *Ann Hum Genet* 2011; **75**: 255-265 [PMID: 21241273 DOI: 10.1111/j.1469-1809.2010.00634.x]

4 **Albus C**. Psychological and social factors in coronary heart disease. *Ann Med* 2010; **42**: 487-494 [PMID: 20839918 DOI: 10.3109/07853890.2010.515605]

5 **Dent TH**. Predicting the risk of coronary heart disease I. The use of conventional risk markers. *Atherosclerosis* 2010; **213**: 345-351 [PMID: 20637467 DOI: 10.1016/j.atherosclerosis.2010.06.019]

6 **Albandar JM**, Brunelle JA, Kingman A. Destructive periodontal disease in adults 30 years of age and older in the United States, 1988-1994. *J Periodontol* 1999; **70**: 13-29 [PMID: 10052767 DOI: 10.1902/jop.1999.70.1.13]

7 **Pihlstrom BL**, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005; **366**: 1809-1820 [PMID: 16298220 DOI: 10.1016/S0140-6736(05)67728-8]

8 **Cullinan MP**, Ford PJ, Seymour GJ. Periodontal disease and systemic health: current status. *Aust Dent J* 2009; **54** Suppl 1: S62-S69 [PMID: 19737269 DOI: 10.1111/j.1834-7819.2009.01144.x]

9 **Blaizot A**, Vergnes JN, Nuwwareh S, Amar J, Sixou M. Periodontal diseases and cardiovascular events: meta-analysis of observational studies. *Int Dent J* 2009; **59**: 197-209 [PMID: 19774803]

10 **Preshaw PM**, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, Taylor R. Periodontitis and diabetes: a two-way relationship. *Diabetologia* 2012; **55**: 21-31 [PMID: 22057194 DOI: 10.1007/s00125-011-2342-y]

11 **Zeng XT**, Tu ML, Liu DY, Zheng D, Zhang J, Leng W. Periodontal disease and risk of chronic obstructive pulmonary disease: a meta-analysis of observational studies. *PLoS One* 2012; **7**: e46508 [PMID: 23094025 DOI: 10.1371/journal.pone.0046508]

12 **Lowe GD**. The relationship between infection, inflammation, and cardiovascular disease: an overview. *Ann Periodontol* 2001; **6**: 1-8 [PMID: 11887452 DOI: 10.1902/annals.2001.6.1.1]

13 **Li YG**, Li WL. [Assessing the relationship between periodontal infection and coronary heart disease in elderly people]. *Linchuang Kouqiang Yixue Zazhi* 2001; **17**: 15-16

14 **López R**, Oyarzún M, Naranjo C, Cumsille F, Ortiz M, Baelum V. Coronary heart disease and periodontitis -- a case control study in Chilean adults. *J Clin Periodontol* 2002; **29**: 468-473 [PMID: 12060431 DOI: 10.1034/j.1600-051X.2002.290513.x]

15 **Huang HM**, Wang QT, Yan YP. [A clinical questionnaire

about the relationship between the periodontal disease and coronary heart disease]. *Yati Yasui Yazhoubingxue Zazhi* 2003; **13**: 218-221
16 **Liu CL**. [Clinical observation of the relationship between chronic periodontitis and incidence of coronary heart disease]. *Xiandai Kouqiang Yixue Zazhi* 2003; **17**: 374-375
17 **Rutger Persson G**, Ohlsson O, Pettersson T, Renvert S. Chronic periodontitis, a significant relationship with acute myocardial infarction. *Eur Heart J* 2003; **24**: 2108-2115 [PMID: 14643271 DOI: 10.1016/j.ehj.2003.10.007]
18 **Geerts SO**, Legrand V, Charpentier J, Albert A, Rompen EH. Further evidence of the association between periodontal conditions and coronary artery disease. *J Periodontol* 2004; **75**: 1274-1280 [PMID: 15515345 DOI: 10.1902/jop.2004.75.9.1274]
19 **Montebugnoli L**, Servidio D, Miaton RA, Prati C, Tricoci P, Melloni C. Poor oral health is associated with coronary heart disease and elevated systemic inflammatory and haemostatic factors. *J Clin Periodontol* 2004; **31**: 25-29 [PMID: 15058371 DOI: 10.1111/j.0303-6979.2004.00432.x]
20 **Renvert S**, Ohlsson O, Persson S, Lang NP, Persson GR. Analysis of periodontal risk profiles in adults with or without a history of myocardial infarction. *J Clin Periodontol* 2004; **31**: 19-24 [PMID: 15058370 DOI: 10.1111/j.0303-6979.2004.00431.x]
21 **Tang LY**, Huang W. [Study on the relationship between peridontal disease and coronary heart disease]. *Huaxia Yixue* 2004; **17**: 959-960
22 **Buhlin K**, Gustafsson A, Ahnve S, Janszky I, Tabrizi F, Klinge B. Oral health in women with coronary heart disease. *J Periodontol* 2005; **76**: 544-550 [PMID: 15857094 DOI: 10.1902/jop.2005.76.4.544]
23 **Liu P**, Wang SJ, Zhang Y, Zhang CH, Zheng PH. [The incidence of peridontal disease is higher among coronary heart disease population]. *Zhongguo Dongmai Yinghua Zazhi* 2005; **13**: 36
24 **Wang J**, Liu CL, Zhang SC, Zheng G. [The influence of chronic periodontitis on coronary heart disease]. *Zhongguo Manxingbing Yufang Yu Kongzhi* 2005; **13**: 27-28
25 **Andriankaja OM**, Genco RJ, Dorn J, Dmochowski J, Hovey K, Falkner KL, Scannapieco F, Trevisan M. The use of different measurements and definitions of periodontal disease in the study of the association between periodontal disease and risk of myocardial infarction. *J Periodontol* 2006; **77**: 1067-1073 [PMID: 16734583 DOI: 10.1902/jop.2006.050276]
26 **Barilli AL**, Passos AD, Marin-Neto JA, Franco LJ. Periodontal disease in patients with ischemic coronary atherosclerosis at a University Hospital. *Arq Bras Cardiol* 2006; **87**: 695-700 [PMID: 17262105 DOI: 10.1590/S0066-782X2006001900003]
27 **Briggs JE**, McKeown PP, Crawford VL, Woodside JV, Stout RW, Evans A, Linden GJ. Angiographically confirmed coronary heart disease and periodontal disease in middle-aged males. *J Periodontol* 2006; **77**: 95-102 [PMID: 16579709 DOI: 10.1902/jop.2006.77.1.95]
28 **Geismar K**, Stoltze K, Sigurd B, Gyntelberg F, Holmstrup P. Periodontal disease and coronary heart disease. *J Periodontol* 2006; **77**: 1547-1554 [PMID: 16945033 DOI: 10.1902/jop.2006.050405]
29 **Li GZ**, Xu L, Quan ZL, Kou Z, Cai LN, Su LZ, Li XY, Zhong YF. [Study of the relationship between periodotal disease and coronary heart disease in elderly people]. *Nanfang Yike Daxue Xuebao* 2006; **26**: 1652-1654
30 **Spahr A**, Klein E, Khuseyinova N, Boeckh C, Muche R, Kunze M, Rothenbacher D, Pezeshki G, Hoffmeister A, Koenig W. Periodontal infections and coronary heart disease: role of periodontal bacteria and importance of total pathogen burden in the Coronary Event and Periodontal Disease (CORODONT) study. *Arch Intern Med* 2006; **166**: 554-559 [PMID: 16534043]
31 **Zhang DJ**, Liu J, Zhao XZ. [Research of the relationship between the periodontal and coronary heart disease]. *Yati Yasui Yazhoubingxue Zazhi* 2006; **16**: 38-40
32 **Zhang YM**, Zhong LJ, He BX, Nie J, Wang X, Li WC. [Study on the correlation between coronary heart disease and chronic periodontitis]. *Zhonghua Liuxingbingxue Zazhi* 2006; **27**: 256-259 [PMID: 16792902]
33 **Latronico M**, Segantini A, Cavallini F, Mascolo A, Garbarino F, Bondanza S, Debbia EA, Blasi G. Periodontal disease and coronary heart disease: an epidemiological and microbiological study. *New Microbiol* 2007; **30**: 221-228 [PMID: 17802899]
34 **Nonnenmacher C**, Stelzel M, Susin C, Sattler AM, Schaefer JR, Maisch B, Mutters R, Flores-de-Jacoby L. Periodontal microbiota in patients with coronary artery disease measured by real-time polymerase chain reaction: a case-control study. *J Periodontol* 2007; **78**: 1724-1730 [PMID: 17760542 DOI: 10.1902/jop.2007.060345]
35 **Rech RL**, Nurkin N, da Cruz I, Sostizzo F, Baião C, Perrone JA, Wainstein R, Pretto D, Manenti ER, Bodanese LC. Association between periodontal disease and acute coronary syndrome. *Arq Bras Cardiol* 2007; **88**: 185-190 [PMID: 17384836 DOI: 10.1590/S0066-782X2007000200009]
36 **Ge S**, Wu YF, Liu TJ, Meng S, Zhao L. [Study of the correlation between moderately and severely chronic periodontitis and coronary heart disease]. *Huaxi Kouqiang Yixue Zazhi* 2008; **26**: 262-266 [PMID: 18705507]
37 **Meng PS**, Wei ZM, Bai XF, Li ZQ. [Research of the Relationship between the Periodontal Disease and Coronary Heart Disease]. *Zhongguo Yiyao Daokan* 2008; **10**: 486-487
38 **Wu ZZ**, Yao XL. [Study the association between Periodontal Disease and coronary atherosclerotic heart disease]. *Xiandai Yixue* 2008; **36**: 185-187
39 **Zamirian M**, Raoofi S, Khosropanah H, Javanmardi R. Relationship Between Periodontal Disease and Acute Myocardial Infarction. *ICRJ* 2008; **1**: 216-221
40 **Zhu L**, Yan LY. [Investgation of peridontal status of community population with diabetes mellitus or myocardial infarction]. *Zhongxiyi Jiehe Xinnaoxueguanbing Zazhi* 2008; **6**: 1118-1120
41 **Dong LW**, Xing CY, Wang XH, Huang W. [Research of the relationship between the periodontal disease and coronary heart disease]. *Guangdong Yabing Fangzhi* 2009; **17**: 357-361
42 **Ma HW**, Li F. [Study of relationship between periodontal disease and coronary heart disease]. *Shangdong Yiyao* 2009; **49**: 76-78
43 **Oikarinen K**, Zubaid M, Thalib L, Soikkonen K, Rashed W, Lie T. Infectious dental diseases in patients with coronary artery disease: an orthopantomographic case-control study. *J Can Dent Assoc* 2009; **75**: 35 [PMID: 19239740]
44 **Sun SY**, Jin AM, Fan WH, Zhang JC. [Evalution of peridontal status in patients with coronary heart disease]. *Nanfang Yike Daxue Xuebao* 2009; **29**: 144-147
45 **Willershausen B**, Kasaj A, Willershausen I, Zahorka D, Briseño B, Blettner M, Genth-Zotz S, Münzel T. Association between chronic dental infection and acute myocardial infarction. *J Endod* 2009; **35**: 626-630 [PMID: 19410072 DOI: 10.1016/j.joen.2009.01.012]
46 **Bokhari SA**, Khan AA, Khalil M, Abubakar MM, Mustahsen-U-Rehaman M. Oral health status of CHD and non-CHD adults of Lahore, Pakistan. *J Indian Soc Periodontol* 2011; **15**: 51-54 [PMID: 21772722 DOI: 10.4103/0972-124X.82273]
47 **Chen JX**. [Investigation and analysis of relationship between periodontitis and coronary heart disease]. *Zhongguo Shequ Yishi* 2011; **13**: 163
48 **Sikka M**, Sequeira PS, Acharya S, Bhat M, Rao A, Nagaraj A. Poor oral health in patients with coronary heart disease: a case-control study of Indian adults. *N Z Med J* 2011; **124**: 53-62 [PMID: 22237568]
49 **Ashraf J**, Hussain Bokhari SA, Manzoor S, Khan AA. Poor oral health and coronary artery disease: a case-control study. *J Periodontol* 2012; **83**: 1382-1387 [PMID: 22324468 DOI: 10.1902/jop.2012.110563]
50 **Zhang LP**, Li XQ, Zhou GX, Fan HM. [Periodontal disease

was an independent risk factor of coronary heart disease by multi-factors Logistic regression analysis]. *Zhongguo Zonghe Linchuang* 2012; **28**: 284-286 [DOI: 10.3760/cma.j.issn.1008-6315.2012.03.022]

51 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670 DOI: 10.1001/jama.283.15.2008]

52 **Wells GA**, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. *JCO* 2010; **28**: e121 [DOI: 10.1200/JCO.2009.26.7443]

53 **Borenstein M**, Hedges L, Higgins J, Rothstein H. Comprehensive Meta-analysis. 2nd ed. Englewood, NJ: BioStat, 2005

54 **Higgins JP**, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558 [PMID: 12111919 DOI: 10.1002/sim.1186]

55 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]

56 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]

57 **Duval S**, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000; **56**: 455-463 [PMID: 10877304 DOI: 10.1111/j.0006-341X.2000.00455.x]

58 **Mattila KJ**, Nieminen MS, Valtonen VV, Rasi VP, Kesäniemi YA, Syrjälä SL, Jungell PS, Isoluoma M, Hietaniemi K, Jokinen MJ. Association between dental health and acute myocardial infarction. *BMJ* 1989; **298**: 779-781 [PMID: 2496855 DOI: 10.1136/bmj.298.6676.779]

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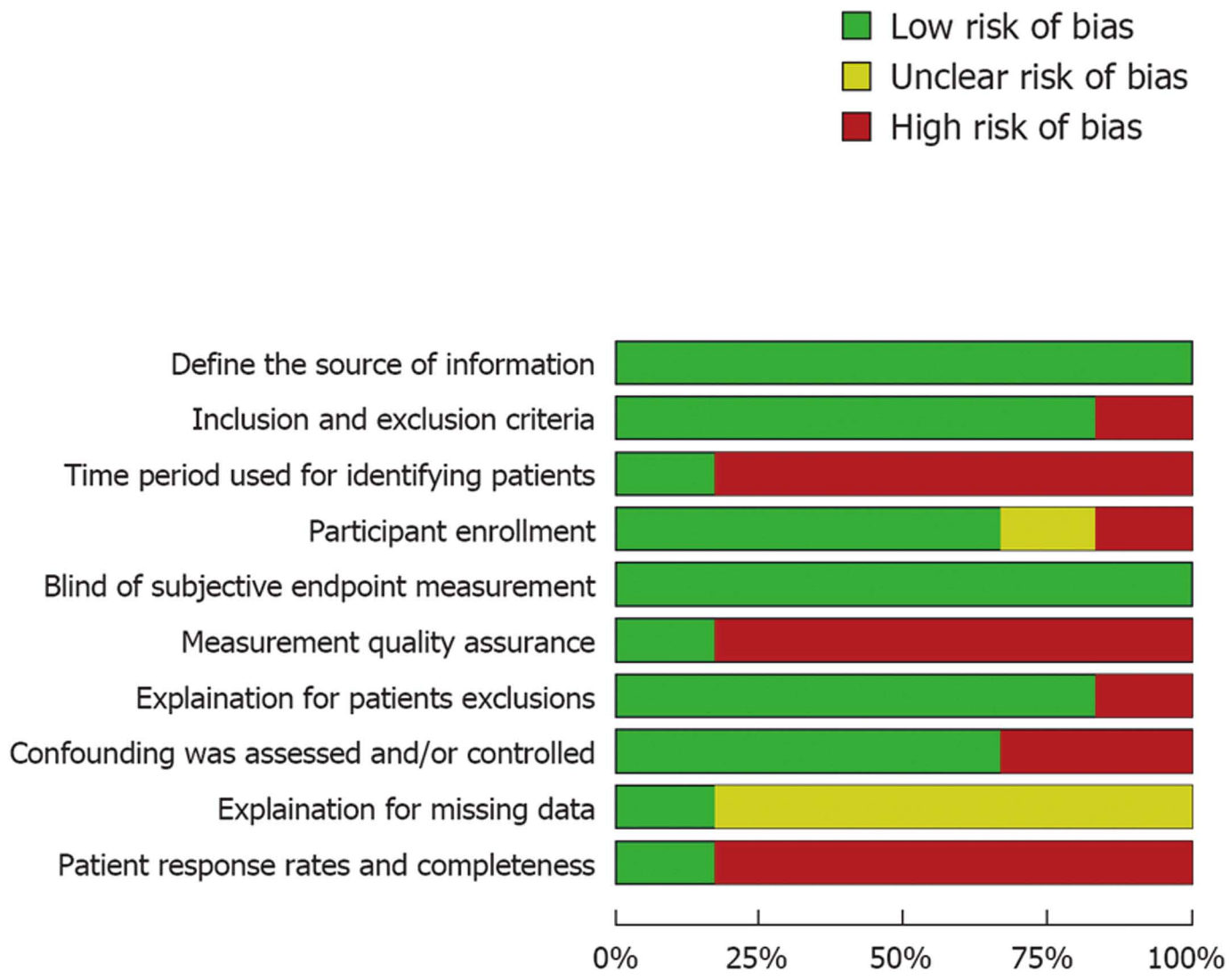

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META-ANALYSIS

Dose-response relationship of lung cancer to amount smoked, duration and age starting

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Author contributions: Lee PN, Fry JS and Forey BA planned the study; Literature searches were carried out by Coombs KJ, assisted by Lee PN and Forey BA; Data entry was carried out by Coombs KJ and checked by Forey BA, or carried out by Forey BA and checked by Lee PN; Where appropriate, difficulties in interpreting published data or in the appropriate methods for derivation of RRs were discussed by Forey BA and Lee PN; The statistical analyses were conducted by Fry JS along lines discussed and agreed with Lee PN; Lee PN and Fry JS jointly drafted the paper, which was critically reviewed by Forey BA and Coombs KJ.
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Abstract

AIM: To quantify smoking/lung cancer relationships accurately using parametric modelling.

METHODS: Using the International Epidemiological Studies on Smoking and Lung Cancer database of all epidemiological studies of 100+ lung cancer cases published before 2000, we analyzed 97 blocks of data for amount smoked, 35 for duration of smoking, and 27 for age started. Pseudo-numbers of cases and controls (or at risk) estimated from RRs by dose level formed the data modelled. We fitted various models relating $\log_e$ RR to dose (d), including βd, βd^{γ} and $\beta \log_e (1 + \omega d)$, and investigated goodness-of-fit and heterogeneity between studies.

RESULTS: The best-fitting models for $\log_e$ RR were $0.833 \log_e [1 + (8.1c/10)]$ for cigarettes/d (c), $0.792 (y/10)^{0.74}$ for years smoked (y) and $0.176 [(70 - a)/10]^{1.44}$ for age of start (a). Each model fitted well overall, though some blocks misfitted. RRs rose from 3.86 to 22.31 between c = 10 and 50, from 2.21 to 13.54 between y = 10 and 50, and from 3.66 to 8.94 between a = 30 and 12.5. Heterogeneity ($P < 0.001$) existed by continent for amount, RRs for 50 cigarettes/d being 7.23 (Asia), 26.36 (North America) and 22.16 (Europe). Little heterogeneity was seen for duration of smoking or age started.

CONCLUSION: The models describe the dose-relationships well, though may be biased by factors including misclassification of smoking status and dose.

Key words: Smoking; Lung neoplasms; Dose-response; Meta-analysis; Review; Amount smoked; Duration of smoking; Age at starting to smoke

Core tip: This paper, for the first time, meta-analyses smoking/lung cancer dose-relationships. Based on data from 71 studies published before 2000, single parameter models were fitted to summarize how the RR increased with increasing amount smoked, longer duration of smoking, and earlier age of starting to smoke. Overall, the models fitted well. Little heterogeneity was seen for duration of smoking or age of start, but the rise in RR with amount smoked was much steeper in North America and Europe than in Asia. The fitted models can be used to more precisely estimate the lung cancer risk from smoking.

Fry JS, Lee PN, Forey BA, Coombs KJ. Dose-response relationship of lung cancer to amount smoked, duration and age starting. *World J Meta-Anal* 2013; 1(2): 57-77 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i2/57.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i2.57

INTRODUCTION

We recently carried out a systematic review[1] of the evidence relating smoking to lung cancer incorporating all 287 studies published before 2000 involving a minimum of 100 lung cancer cases. We refer to this as "our earlier review". In that review, we assessed evidence concerning amount smoked per day, duration of smoking, and age of starting to smoke. Data are typically available as blocks of RRs for differing levels of the dose-response measure, each compared to never smokers. Comparing meta-analysis estimates for low, medium and high exposure, we clearly demonstrated a dose-response existed. For example, for amount smoked by current smokers, random-effects RR estimates are 4.71 (95%CI: 4.14-5.37, n = 86) for about 5 cigs/d, 9.83 (95%CI: 8.60-11.24, n = 54) for about 20 cigs/d, and 17.10 (95%CI: 14.62-19.99, n = 62) for about 45 cigs/d. Here "about 5 cigs/d" combined results for dose ranges including 5 but not 20 cigs/d, "about 20 cigs/d" considered ranges including 20 but not 5 or 45 cigs/d, and "about 45 cigs/d" ranges including 45 but not 20 cigs/d. This approach has limitations. First, formal statistical comparison of the RRs at the different levels is not possible as the RRs are not independent, having the same denominator. Second, the analyses do not use all the information available. Thus, results for ranges wholly between 5 and 20 cigs/d or wholly between 20 and 45 cigs/d are ignored, as are results for ranges covering two or more of the "key values" of 5, 20 and 45 cigs/d. Also, linearity, or other shapes of the relationship, is not assessed. Dose-response relationships for years quit are considered in a separate paper[2].

Here, we study dose-response in more detail by fitting models to the various dose-response blocks to estimate parameters which can be meta-analyzed and used to assess heterogeneity. We follow the approach previously used[3] to quantify the dose-response relationship between environmental tobacco smoke exposure and lung cancer risk, developing a variant of it for age of starting. We restrict attention to the data considered in our earlier review[1]. Rather than also considering results for ever smokers, we restrict attention to current smokers, giving a more homogeneous dataset and one showing a stronger dose-relationship. All our analyses are of overall lung cancer risk, no attempt being made in the present paper to fit models for specific histological types.

MATERIALS AND METHODS

The International Evidence on Smoking and Lung Cancer database

All analyses use the International Evidence on Smoking and Lung Cancer database, fully described in our earlier review[1]. Papers considered were published before 2000, described studies of 100+ cases, and provided RR estimates for one or more smoking indices. We use the term RR generically to describe alternative RR estimates, *e.g.*, odds ratio or hazard ratio. Lee *et al*[1] gives details of the structure and data entry rules for the database.

Data selection and blocks

The data considered here comprise blocks of RRs, each relative to never smokers, for all lung cancer (or occasionally near equivalent definitions, each including squamous cell carcinoma and adenocarcinoma) for three measures of dose-response among current smokers: amount smoked, duration and age of starting. Where possible, blocks by sex or by sex and race were considered. Except for amount smoked, blocks by age were considered, if available. Covariate-adjusted RRs were preferred to unadjusted RRs. Each block includes an estimate of the RR and 95%CI: for each level of the measure. The data recorded per block included study type, sex, location, publication year, age range (at baseline for prospective studies), product smoked [any product, cigarettes +/- other products (*i.e.*, pipes, cigars), cigarettes only], never smoker definition (never any product, never cigarettes). For each RR, the range of the measure was also recorded.

Pseudo-numbers

We used the method of Hamling *et al*[4] on each block to estimate the pseudo-table of numbers of cases, and either controls (for case-control studies) or at risk (for prospective studies) which correspond to the observed RRs and 95%CIs. The method was applied even to unadjusted RRs. This estimation requires, in addition to the given RRs and 95%CIs, estimates of the proportion of never smokers among the controls/at risk and of the ratio of total controls/at risk to total cases, as well as starting values for the numbers of never smoking cases and controls/at risk. These estimates were also recorded on the database. The pseudo-table forms the basic data for fitting the models used, and estimating the overall current smoking RR.

Midpoints for levels of exposure

For amount smoked, midpoint estimates for each exposure level were derived using standard distributions, as described by Fry *et al*[3] when relating lung cancer risk to amount smoked by the husband. For US studies, the distribution derived from published data for two large CPS I and CPS II studies[5], while for non-US studies, it was that given in Appendix III of International Smoking Statistics[6,7].

For duration of smoking and age of starting the midpoints were based on US NHANES III[8], selecting data for subjects for the given sex, age and range of values of the relevant dose-response measure.

Statistical models

For each measure, the data analyzed consist of blocks, each containing the pseudo-numbers and the estimated midpoint exposures for each of ℓ exposure levels, and for never smokers. The methodology varies by dose-response measure, as described below.

Table 1 Models used to relate risk to dose

$\log_e$ (RR)	=	$\beta_1 d$	(linear)
$\log_e$ (RR)	=	$\beta_1 d + \beta_2 d^2$	(quadratic)
$\log_e$ (RR)	=	$\beta_1 d + \beta_2 d^2 + \beta_3 d^3$	(cubic)
$\log_e$ (RR)	=	$\beta_1 d^Y$	(power)
$\log_e$ (RR)	=	$\beta_1 \log_e$ (d)	(log)
$\log_e$ (RR)	=	$\beta_1 \exp_e$ (d)	(exponential)
$\log_e$ (RR)	=	$\beta_1 \log_e$ (1 + Wd)	(log-with-baseline)

Amount smoked

The Greenland and Longnecker method[9,10] was used to fit functional forms relating RR to dose (midpoint amount smoked). We fitted the models expressing dose, d, in units of 10 cigs/d.

In the simplest application, the RR is predicted by $\log_e$ RR (d) = βd, β and SE (β) are estimated separately per block, and estimates of β and SE (β) are then combined using inverse-variance weighted random-effects or fixed-effects meta-analysis[11]. This model implies that a fixed dose increment increases risk by a fixed factor. The method can be used with d replaced by a function of d, such as $d^{1/2}$, d^2, or log (d + 1).

Greenland *et al*[9] describe a more general, "pool-first", method in which all the blocks are considered in a single analysis. The method gives the same results for the model log RR = βd, but allows direct fitting for other functional forms.

As the best model was initially unclear, we first tried various models (Table 1) using the pool-first method, comparing deviances to assess which models fitted the overall data better. For the "power" and "log-with-baseline" models, the parameters Y or W could not be fitted directly, but an iterative method was adopted, comparing deviances for a range of values.

For the models with lowest deviance, the simpler approach was then used to estimate β_1 (and β_2, β_3) and its standard error (SE) for each block. For a particular model, goodness-of-fit for a block was tested by comparing observed and fitted number of cases (and for case-control studies also the observed number of controls) at each level of amount smoked (including never smokers). The fitted values were estimated as described in Goodness of fit[11]. As also described there, the sum of (observed - fitted)^2/fitted over levels was taken as an approximate chi-squared on ℓ - 1 degrees of freedom (df) for prospective studies or on 2ℓ - 1 df for case-control studies. Information on overall goodness-of-fit was derived by summing observed and fitted values over blocks for never smokers and for specified levels of amount smoked, and similarly deriving an approximate chi-squared statistic. Plots of observed and predicted RRs per block were also examined.

Duration of smoking

The approach used was as for amount smoked.

Age of starting to smoke

For smokers of a given age, age of starting (a) and duration (y) are directly related. We used the same basic approach, replacing y by 70 - a to produce a duration-like measure. As this produced a relatively good fit, we did not attempt sensitivity analyses replacing y by 60 - a or 80 - a.

Regression analyses

Sources of heterogeneity were studied by inverse-variance weighted regression of β. Between block variation was examined one factor at a time (simple regression), and using forward stepwise methods. The factors used were study type, sex, location, publication year, midpoint age (at baseline for prospective studies), smoking product and study size. The deviance of the fitted models indicated the extent to which heterogeneity was explained.

Statistical analysis

No multiple testing adjustments were made, significant being defined as $P < 0.05$. However, results showing stronger evidence of a relationship ($P < 0.01$, or $P < 0.001$), and sometimes weaker evidence ($P < 0.1$) are also distinguished, where appropriate. All data entry and most statistical analyses were carried out using ROELEE version 3.1 (available from PN Lee Statistics and Computing Ltd., 17 Cedar Road, Sutton, Surrey SM2 5DA, United Kingdom). Some analyses used Excel 2003.

RESULTS

Studies considered

For each of the 71 studies providing the data used, Studies[11], gives the six character reference code (REF); a brief description incorporating the location, characteristics of the population studied, study design, and study duration; the total number of lung cancers studied; and the measures for which data are analyzed.

Amount smoked

Details of blocks used for each measure are given in Blocks[11]. These include study REF, sex (and where applicable race), study type, location, product smoked, definition of unexposed group, adjustment factors used, current smoker RR, and total numbers of cases in smokers.

The 97 blocks derive from 69 studies, 45 providing results for a single block, 22 results by sex, and 2 (DORGAN and HUMBLE) results by sex and race. 55 blocks (56.7%) are for males, 34 (35.1%) females, and 8 (8.2%) both sexes. 48 (49.5%) are from prospective studies. 43 (44.3%) are from North American studies, 32 (33.0%) from Europe and 17 (17.5%) from Asia, the remaining 5 (5.2%) from South America, Africa or Australasia. Five different combinations of product *vs* unexposed occur: cigarettes ± other products *vs* never any product (32 blocks 33.0%), cigarettes ± other products *vs* never cigarettes (29, 29.9%), any product *vs* never any product (20, 20.6%), cigarettes only *vs* never any product (13, 13.4%) and cigarettes only *vs* never cigarettes for (3, 3.1%). Of the 8 blocks for sexes combined, 4 (50.0%) concern RRs adjusted for sex, while 64 of the full 97 blocks (66.0%)

Table 2 Amount smoked by current smokers (cigarettes per day) - dose-response data

Block: Study	Amount smoked groupings[1]	Mean values	RRs[2]
1: AKIBA	1-14, 15-24, 25+	8.11, 19.19, 34.63	3.50, 6.10, 19.10 M
2: AKIBA	1-14, 15+	8.11, 24.09	3.60, 5.80 M
3: ARCHER	1-19, 20, 21+	10, 20, 31.83	3.53, 6.09, 8.52 M
4: AXELSS	20	20	43.30
5: BENSHL	1-9, 10-19, 20+	4.85, 12.73, 26.03	4.00, 9.05, 10.95 M
6: BEST	1-9, 10-20, 21+	4.85, 15.92, 31.83	10.00, 16.41, 17.31 M
7: BOUCOT	1-20, 21-40, 41+	13.38, 29.02, 53.33	54.09, 78.56, 161.70 M
8: BRETT	1-14, 15-24, 25+	8.11, 19.19, 34.63	2.55, 4.25, 8.00 M
9: BROSS	1-20, 21+	13.38, 31.83	4.91, 7.20 M
10: BUFFLE	1-19, 20, 21+	10.20, 31.83	5.60, 11.84, 22.10 M
11: CEDERL	1-7, 8-15, 16+	4.29, 11.61, 25.41	3.40, 7.50, 11.90 M
12: CEDERL	1-7, 8-15, 16+	4.29, 11.61, 25.41	2.83, 7.74, 7.56
13: CHANG	1-10, 11-20, 21+	7.08, 17.67, 31.83	5.02, 10.60, 8.26
14: CHANG	1-10, 11-20, 21+	7.08, 17.67, 31.83	3.03, 4.87, 8.21 M
15: CHOW	1-19, 20-29, 30+	10, 21.35, 38.39	13.88, 21.87, 44.48 M
16: COMSTO	1-19, 20-39, 40+	10, 22.90, 45.71	12.42, 18.16, 24.92 M
17: COMSTO	1-19, 20-39, 40+	10, 22.90, 45.71	7.45, 17.35, 13.27
18: CORREA	1-20, 21+	13.38, 31.83	9.30, 25.30 M
19: CPSI	1-9, 10-19, 20-39, 40+	4.85, 12.73, 22.90, 45.71	4.51, 8.41, 14.30, 17.49 M
20: CPSII	1-9, 10-19, 20, 21-39, 40, 41+	4.85, 12.73, 20, 26.71, 40, 53.33	12.22, 14.52, 21.59, 22.72, 24.14, 45.52 M
21: CPSII	1-9, 10-19, 20, 21-39, 40, 41+	4.85, 12.73, 20, 26.71, 40, 53.33	3.89, 8.33, 14.21, 21.40, 19.31, 18.22
22: DARBY	1-14, 15-24, 25+	8.11, 19.19, 34.63	73.47, 95.43, 142.69 M
23: DARBY	1-14, 15-24, 25+	8.11, 19.19, 34.63	15.70, 21.50, 41.62 M
24: DEAN3	1-12, 13-22, 23+	7.65, 18.42, 33.04	5.46, 7.42, 21.66 M
25: DEAN3	1-12, 13-22, 23+	7.65, 18.42, 33.04	3.16, 8.42, 24.24 M
26: DEKLER	1-14, 15-24, 25+	8.11, 19.19, 34.63	19.40, 23.00, 32.50 M
27: DOLL2	1-14, 15-24, 25+	8.11, 19.19, 34.63	5.20, 10.60, 22.40 M
28: DOLL2	1-14, 15-24, 25+	8.11, 19.19, 34.63	1.29, 6.43, 29.71 M
29: DORANT	1-9, 10-19, 20+	4.85, 12.73, 26.03	8.52, 27.22, 36.24 M
30: DORGAN	1-19, 20+	10, 26.03	9.13, 20.65 M
31: DORGAN	1-19, 20+	10, 26.03	26.67, 72.46 M
32: DORGAN	1-19, 20+	10, 26.03	6.55, 24.13 M
33: DORGAN	1-19, 20+	10, 26.03	7.43, 41.43 M
34: DORN	1-9, 10-20, 21-39, 40+	4.85, 15.92, 26.71, 45.71	4.02, 9.92, 17.19, 22.75 M
35: ENGELA	1-4, 5-9, 10-14, 15-19, 20+	2.5, 6.5, 10.88, 15.83, 26.03	1.40, 4.10, 7.00, 11.00, 15.00 M
36: ENGELA	1-4, 5-9, 10-14, 15+	2.5, 6.5, 10.88, 24.09	12.00, 12.00, 24.00, 26.00
37: ENSTRO	1-9, 10-19, 20, 21-39, 40+	4.85, 12.73, 20, 26.71, 45.71	4.74, 7.68, 13.65, 16.08, 19.41 M
38: ENSTRO	1-9, 10-19, 20, 21+	4.85, 12.73, 20, 31.83	2.15, 4.31, 9.48, 16.47 M
39: GAO2	1-19, 20-29, 30+	10, 21.35, 38.39	3.36, 7.54, 10.63 M
40: GILLIS	1-14, 15-24, 25-34, 35-49, 50+	8.11, 19.19, 28.13, 39, 53.33	4.50, 7.60, 8.60, 9.70, 7.80
41: HAENSZ	1-20, 21+	13.38, 31.83	1.77, 5.15 M
42: HAMMO2	1-19, 20+	10.00, 26.03	9.15, 10.39 M
43: HAMMON	1-9, 10-20, 21-39, 40+	4.85, 15.92, 26.71, 45.71	7.44, 8.42, 17.91, 20.64 M
44: HIRAYA	1-9, 10-19, 20+	4.85, 12.73, 26.03	2.06, 4.00, 6.24 M
45: HIRAYA	1-9, 10-19, 20+	4.85, 12.73, 26.03	2.25, 2.56, 4.47 M
46: HITOSU	1-14, 15-24, 25+	8.11, 19.19, 34.63	2.08, 2.82, 4.68 M
47: HITOSU	1-14, 15+	8.11, 24.09	3.11, 3.17 M
48: HOLE	1-14, 15-24, 25-34, 35+	8.11, 19.19, 28.13, 44.38	5.47, 8.90, 10.75, 7.49
49: HUMBLE	1-19, 20+	10, 26.03	9.20, 24.70 M
50: HUMBLE	1-19, 20+	10, 26.03	11.60, 26.10 M
51: HUMBLE	1-19, 20+	10, 26.03	19.20, 16.00
52: HUMBLE	1-19, 20+	10, 26.03	18.50, 36.90 M
53: KAISE2	1-19, 20+	10, 26.03	4.47, 10.34 M
54: KAISE2	1-19, 20+	10, 26.03	7.61, 22.12 M
55: KAISER	1-19, 20-40, 41+	10, 24.32, 53.33	6.58, 17.24, 20.91 M
56: KAISER	1-19, 20-40, 41+	10, 24.32, 53.33	3.42, 7.98, 12.63 M
57: KANELL	1-10, 11-20, 21-35, 36+	7.08, 17.67, 26.71, 45.71	1.71, 7.06, 20.39, 34.22 M
58: KATSOU	1-20, 21+	13.38, 31.83	2.26, 7.46 M
59: KAUFMA	1-14, 15-24, 25-34, 35-44, 45+	8.11, 19.19, 28.13, 39, 53.33	8.00, 15.00, 28.00, 43.00, 60.00 M
60: KINLEN	1-14, 15-24, 25+	8.11, 19.19, 34.63	10.61, 14.14, 21.74 M
61: KNEKT	1-14, 15+	8.11, 24.09	5.00, 12.70 M
62: KOO	1-10, 11-20, 21-30	7.08, 17.67, 25.88	1.36, 7.29, 1.52
63: LIAW	1-10, 11-20, 21+	7.08, 17.67, 31.83	3.10, 3.60, 8.30 M
64: LIDDEL	1-19, 20+	10, 26.03	3.33, 5.02 M
65: MACLEN	1-9, 10-19, 20-29, 30+	4.85, 12.73, 21.35, 38.39	1.36, 3.41, 4.16, 5.00 M
66: MACLEN	1-9, 10-19, 20+	4.85, 12.73, 26.03	0.76, 3.44, 3.84
67: MATOS	1-14, 15-24, 25+	8.11, 19.19, 34.63	1.60, 8.00, 15.00 M

68: MIGRAN	1-9, 10-19, 20, 21+	4.85, 12.73, 20, 31.83	4.01, 4.24, 5.14, 5.93 M
69: MIGRAN	1-9, 10-19, 20	4.85, 12.73, 20	4.88, 6.53, 7.48 M
70: MRFITR	1-19, 20-39, 40+	10, 22.90, 45.71	10.86, 50.12, 56.43 M
71: NAM	1-24, 25+	14.06, 34.63	6.70, 10.27 M
72: NAM	1-24, 25+	14.06, 34.63	9.06, 16.65 M
73: PARKIN	1-14, 15+	8.11, 24.09	3.90, 5.20 M
74: PERSH2	1-9, 10+	4.85, 20.90	5.76, 11.34 M
75: PETO	1-14, 15+	8.11, 24.09	5.50, 9.49 M
76: PEZZO2	1-20, 21-40, 41+	13.38, 29.02, 53.33	8.00, 44.39, 112.13 M
77: PEZZOT	1-20, 21-40, 41+	13.38, 29.02, 53.33	7.40, 70.00, 246.50 M
78: PRESCO	1-14, 15+	8.11, 24.09	10.20, 19.96 M
79: PRESCO	1-14, 15+	8.11, 24.09	6.36, 10.08 M
80: SEGI2	1-9, 10-19, 20-29, 30-39, 40+	4.85, 12.73, 21.33, 31.07, 45.71	2.10, 3.10, 3.40, 6.90, 7.90 M
81: SEGI2	1-9, 10-19, 20+	4.85, 12.73, 26.03	2.90, 1.44, 1.03
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84: SPEIZE	1-4, 5-14, 15-24, 25-34, 35+	2.5, 9.42, 19.19, 28.13, 44.38	2.70, 5.20, 12.60, 15.70, 22.00 M
85: STOCKW	1-19, 20-40, 41+	10, 24.32, 53.33	6.67, 14.51, 28.84 M
86: SVENSS	1-10, 11-20, 21+	7.08, 17.67, 31.83	4.60, 12.60, 59.00 M
87: TENKAN	1-14, 15-24, 25+	8.11, 19.19, 34.63	15.86, 20.25, 24.97 M
88: TSUGAN	1-15, 16-35, 36+	9.33, 22.45, 45.71	0.90, 1.22, 1.66
89: TULINI	1-14, 15-24, 25+	8.11, 19.19, 34.63	6.02, 12.00, 27.30 M
90: TULINI	1-14, 15-24, 25+	8.11, 19.19, 34.63	8.17, 26.30, 38.70 M
91: TVERDA	1-9, 10-19, 20+	4.85, 12.73, 26.03	2.14, 3.32, 6.56 M
92: TVERDA	1-9, 20+	4.85, 26.03	4.53, 18.00 M
93: WAKAI	1-19, 20-20, 30+	10.00, 21.35, 38.39	1.80, 4.01, 9.19 M
94: WU	1-20, 21+	13.38, 31.83	3.25, 8.48 M
95: WYNDE6	1-10, 11-20, 21-30, 31+	7.08, 17.67, 25.88, 43.06	6.80, 11.16, 17.32, 28.22 M
96: WYNDE6	1-10, 11-20, 21-30, 31+	7.08, 17.67, 25.88, 38.39	3.75, 11.97, 21.64, 39.14 M
97: YAMAGU	1-20, 21+	13.38, 31.83	3.75, 12.14 M

[1]In some studies, amount smoked is based on cigarette equivalents for cigars and pipes; [2]M indicates a strictly monotonic rise in RR with increasing amount smoked.

concern age-adjusted RRs. Race and/or other factors were adjusted for in 28 (28.9%) blocks.

Table 2 gives for each block the levels used to categorize amount smoked and the corresponding estimated mean values and RRs for each level. The RRs reveal an obvious trend for risk to rise with amount smoked. Of the 96 blocks with more than one level, 84 (87.5%) show a strictly monotonic increase in RR. However, considerable variation is evident in the RR for the highest exposure.

Table 3 gives the pool-first results investigating model suitability. The exponential model is particularly poor, explaining only 21.75% of the overall deviance in the estimates of log RR. The log model is also relatively poor. The linear, quadratic and cubic models are better. However, despite involving more parameters, the cubic model explains less of the overall deviance than do the best-fitting power or log-with-baseline models. The residual deviance is lowest for the log-with-baseline model, the best-fitting W value explaining 94.12% of the overall deviance, though the best-fitting power model explains almost as much (93.95%).

Fit Amount Smoked[11], gives full details for the further analyses carried out using the linear, and best-fitting power and log-with-baseline models. These include 95%CIs for the RRs in Table 2, and observed and fitted numbers by level for each block.

For each of these models, Table 4 compares the observed and fitted numbers of cases summed over blocks for never smokers and for current smokers by amount smoked. The linear model fits poorly, overestimating cases for never smokers and 30+ cigs/d smokers and underestimating for 1-30 cigs/d smokers, the model implying a far steeper increase with amount smoked than observed. This is consistent with the block-specific goodness-of-fit tests, 63 showing misfits significant at $P < 0.05$. This model is clearly inadequate for amount smoked.

Although Table 4 shows highly significant ($P < 0.001$) misfit to both the power and log-with-baseline models, the misfit is not substantial, with observed and expected numbers generally agreeing to a few percent.

For each block, and both models, Table 5 gives fitted values of β_1 and SE and goodness-of-fit P values. A number of blocks show significant ($P < 0.05$) misfit, these tending to be the same blocks for both models. We comment on those 15 blocks where the P value for the log-with-baseline model is < 0.01 (Fit Amount Smoked[11] and Table 2 for further details). These divide into various categories. Three blocks (19: CPS I , 34: DORN, 37: ENSTRO males) involve very large numbers of cases (Table 3) where the model appears to fit quite well, though in block 19: CPS I the observed flattening of response for 40+ cigs/d is not well fitted. Seven blocks (6: BEST, 20: CPS Ⅱ males, 22: DARBY males, 43: HAMMON, 60: KINLEN, 74: PERSH2, 87: TENKAN) show a marked risk increase for the lowest level of amount smoked, but the slope subsequently flattens. In contrast the reverse is true for five blocks (24: DEAN3 males, 38: ENSTRO fe-

Table 3 Comparing the suitability of different models relating log *RR* to amount smoked by current smokers, expressed as d = cigarettes per day/10

Model	Parameter value[1]	Fitted coefficient(s) (SE)	Deviance	DF	Deviance explained (%)
Null	-	-	24894.53	97	
Linear: log RR = $\beta_1 d$	-	β_1 = 0.6107 (0.0046)	7265.32	96	70.82
Quadratic: log RR = $\beta_1 d + \beta_2 d^2$	-	β_1 = 1.4121 (0.0130), β_2 = -0.1792 (0.0027)	2907.39	95	88.32
Cubic: log RR = $\beta_1 d + \beta_2 d^2 + \beta_3 d^3$	-	β_1 = 2.1915 (0.0266), β_2 = -0.6346 (0.0138), β_3 = 0.0633 (0.0019)	1779.05	94	92.86
Power: log RR = $\beta_1 d^Y$	Y = 0.32	β_1 = 1.8922 (0.0124)	1512.49	96	
	Y = 0.33	β_1 = 1.8691 (0.0122)	1506.19	96	
	Y = 0.34	β_1 = 1.8457 (0.0121)	1506.07	96	93.95
	Y = 0.35	β_1 = 1.8222 (0.0119)	1511.92	96	
	Y = 0.36	β_1 = 1.7986 (0.0118)	1523.54	96	
	Y = 0.50	β_1 = 1.4673 (0.0097)	2179.71	96	
	Y = 1.00	β_1 = 0.6107 (0.0046)	7265.32	96	
	Y = 2.00	β_1 = 0.0969 (0.0010)	14739.19	96	
Log: log RR = $\beta_1 \log d$	-	β_1 = 1.2265 (0.0107)	11674.70	96	53.10
Exponential: log RR = $\beta_1 \exp d$	-	β_1 = 0.0120 (0.0002)	19480.21	96	21.75
Log-with-baseline: log RR = $\beta_1 \log (1 + Wd)$	W = 7.5	β_1 = 0.8520 (0.0056)	1466.21	96	
	W = 7.7	β_1 = 0.8456 (0.0055)	1465.37	96	
	W = 7.9	β_1 = 0.8394 (0.0054)	1464.88	96	
	W = 8.0	β_1 = 0.8364 (0.0055)	1464.76	96	
	W = 8.1	β_1 = 0.8334 (0.0054)	1464.71	96	94.12
	W = 8.2	β_1 = 0.8305 (0.0054)	1464.73	96	
	W = 8.3	β_1 = 0.8277 (0.0054)	1464.82	96	

[1]Note that we only sought the best-fitting value of Y to two decimal places and of W to one decimal place.

Table 4 Amount smoked by current smokers - observed and fitted lung cancers for the linear, best power and best log-with-baseline model, with β_1 fitted separately for each block

Midpoint amount smoked (cigs/d)	Observed[1]	Fitted[2]		
		Linear model	Best power model	Best log-with-baseline model
< 5	1249.17	1023.00	1297.42	1173.88
5 to < 10	2579.62	2156.31	2595.37	2539.78
10 to < 15	6125.69	4749.92	6276.74	6299.68
15 to < 20	7940.18	6678.14	8009.06	8156.50
20 to < 30	18138.36	15724.45	17468.99	17678.51
30 to < 40	3858.94	4106.12	3743.23	3701.31
40+	7703.88	8860.42	8115.77	7949.95
Never smoked	6649.61	10947.08	6738.86	6745.84
Total	54245.45	54245.45	54245.45	54245.45
Fit statistic[3]		3792.53	65.29	56.78

[1]Observed pseudo-number of lung cancer cases, summed over blocks; [2]Fitted pseudo-number of lung cancer cases, summed over blocks. For each study, the fitted number of cases for each block is calculated from the numbers at risk and the fitted RRs by amount smoked, derived from the fitted value of β_1; [3]Based on summation of (observed-fitted)^2/fitted, the summation also including terms for the observed and fitted total numbers of controls (not shown). The statistic can be considered to be approximately chisquared on 12 DF and is significant at $P < 0.001$ for all three models.

males, 57: KANELL, 76: PEZZO2, 77: PEZZOT) with the RR for the highest exposure greater than predicted from the response at lower levels. For some of the 15 blocks, the number of cases in never smokers is relatively low (less than 10 in 6 of them) and the best-fitting model gives rather different fitted numbers, so the fitted block of RRs appears substantially different from that observed For example, in block 6: BEST where the observed pseudo-number of cases in never smokers is 6.88, and the observed RRs are 10.00, 16.41 and 17.31 for 1-9, 10-20 and 21+ cigs/d, the fitted number of cases in never smokers is 23.61 and the fitted RRs are 2.47, 4.46 and 6.47.

Table 6 presents results of weighted simple regression analyses of β_1 for the log-with-baseline model. There is highly significant ($P < 0.001$) variation by continent, with β_1 much lower for Asian studies, and by study size, larger studies giving higher β_1 values. Some variation is also seen for sex ($P < 0.05$), study type, publication year and midpoint age ($P < 0.1$), but not with product definition or unexposed group. Table 6 also presents predicted RRs at 20 cigs/d. The variation by continent is clear.

In a forward stepwise analysis (not shown), continent remained highly significant ($P < 0.001$), but no other factor remained significant at $P < 0.05$. The association with study size seems due to a strong correlation with continent.

Table 5 Amount smoked by current smokers - fitted values of β_1 and SE, and *P* values for goodness-of-fit tests for the best-fitting power model and log-with-baseline model

Block: Study	Log-with-baseline model[1] log RR = β_1 [1 + (8.10c/10)]			Power model[1] log RR = β_1 [(c/10)$^{0.34}$]		
	β_1	SE β_1	*P* (fit)[2]	β_1	SE β_1	*P* (fit)[2]
1: AKIBA	0.6599	0.0712	NS	1.4882	0.1609	NS
2: AKIBA	0.6099	0.0660	NS	1.3445	0.1453	NS
3: ARCHER	0.6713	0.1274	NS	1.4932	0.2835	NS
4: AXELSS	1.3245	0.2214	NS	2.9770	0.4976	NS
5: BENSHL	0.7133	0.1169	NS	1.6337	0.2688	NS
6: BEST	0.5678	0.0824	0.0000	1.3483	0.1948	0.0001
7: BOUCOT	0.8332	0.2140	NS	1.7439	0.4482	NS
8: BRETT	0.6637	0.1146	NS	1.4946	0.2575	NS
9: BROSS	0.6090	0.0735	NS	1.3514	0.1634	NS
10: BUFFLE	0.9349	0.1040	NS	2.0810	0.2313	NS
11: CEDERL	0.8191	0.0726	NS	1.8388	0.1634	NS
12: CEDERL	0.7697	0.0693	NS	1.6833	0.1520	NS
13: CHANG	0.6772	0.1522	NS	1.5044	0.3402	NS
14: CHANG	0.6255	0.1165	NS	1.3937	0.2595	NS
15: CHOW	1.0002	0.1050	NS	2.2134	0.2311	NS
16: COMSTO	0.8497	0.1552	NS	1.8491	0.3399	NS
17: COMSTO	0.8857	0.1172	NS	1.9560	0.2601	NS
18: CORREA	0.9945	0.0483	NS	2.2052	0.1069	NS
19: CPS I	0.8314	0.0348	0.0002	1.8278	0.0773	0.0000
20: CPS II	0.8262	0.0331	0.0000	1.8467	0.0739	0.0000
21: CPS II	0.8972	0.0304	0.0153	1.9874	0.0677	0.0006
22: DARBY	0.8198	0.1213	0.0000	1.9085	0.2770	0.0000
23: DARBY	1.0879	0.0841	NS	2.4434	0.1879	NS
24: DEAN3	0.8271	0.0724	0.0009	1.8968	0.1639	0.0042
25: DEAN3	0.8617	0.0801	(0.0569)	1.9152	0.1789	0.0368
26: DEKLER	0.5704	0.1839	NS	1.3114	0.4155	NS
27: DOLL2	1.0464	0.0644	(0.0824)	2.3531	0.1443	NS
28: DOLL2	1.1001	0.1698	NS	2.4424	0.3745	0.0091
29: DORANT	1.1082	0.0867	0.0322	2.5183	0.1972	0.0270
30: DORGAN	0.9728	0.0937	NS	2.1814	0.2101	NS
31: DORGAN	1.4168	0.1835	NS	3.1557	0.4086	NS
32: DORGAN	0.9636	0.0595	NS	2.1418	0.1325	NS
33: DORGAN	1.0359	0.1844	NS	2.2976	0.4094	NS
34: DORN	0.9024	0.0171	0.0001	2.0099	0.0381	0.0000
35: ENGELA	1.0083	0.1270	NS	2.3043	0.2995	NS
36: ENGELA	1.0897	0.1561	0.0206	2.5654	0.3553	NS
37: ENSTRO	0.8261	0.0312	0.0000	1.7910	0.0684	0.0000
38: ENSTRO	0.8229	0.0252	0.0000	1.8368	0.0564	0.0000
39: GAO2	0.7037	0.1042	NS	1.5526	0.2304	NS
40: GILLIS	0.5811	0.0729	NS	1.2704	0.1614	NS
41: HAENSZ	0.3382	0.0805	NS	0.7563	0.1796	NS
42: HAMMO2	0.5198	0.1167	0.0112	1.1870	0.2641	0.0138
43: HAMMON	0.8032	0.0748	0.0038	1.8185	0.1677	0.0116
44: HIRAYA	0.5974	0.0337	NS	1.3374	0.0756	NS
45: HIRAYA	0.4424	0.0501	NS	0.9729	0.1100	NS
46: HITOSU	0.4573	0.1182	NS	1.0324	0.2654	NS
47: HITOSU	0.4988	0.1223	NS	1.0992	0.2686	NS
48: HOLE	0.6142	0.1082	NS	1.3410	0.2413	NS
49: HUMBLE	1.0435	0.1431	NS	2.3386	0.3207	NS
50: HUMBLE	1.0378	0.2633	NS	2.3306	0.5907	NS
51: HUMBLE	0.8922	0.1393	NS	1.9997	0.3114	NS
52: HUMBLE	1.2268	0.2487	NS	2.7318	0.5532	NS
53: KAISE2	0.7599	0.1018	NS	1.6991	0.2278	NS
54: KAISE2	1.0098	0.1107	NS	2.2592	0.2478	NS
55: KAISER	0.7685	0.0477	0.0247	1.6130	0.1010	0.0033
56: KAISER	0.6882	0.0570	NS	1.4932	0.1238	NS
57: KANELL	0.8982	0.0647	0.0000	1.9758	0.1442	0
58: KATSOU	0.4517	0.1260	NS	1.0089	0.2809	NS
59: KAUFMA	1.0712	0.0583	NS	2.3560	0.1278	NS
60: KINLEN	0.6021	0.0616	0.0017	1.3838	0.1399	0.0055
61: KNEKT	0.8644	0.1264	NS	1.9618	0.2876	NS
62: KOO	0.4309	0.1424	NS	0.9299	0.3133	NS
63: LIAW	0.5538	0.0918	NS	1.2384	0.2045	NS
64: LIDDEL	0.5136	0.0742	NS	1.1535	0.1666	NS

65: MACLEN	0.4973	0.1511	NS	1.0875	0.3355	NS
66: MACLEN	0.4301	0.1161	NS	0.9350	0.2577	NS
67: MATOS	0.8315	0.1122	NS	1.8390	0.2485	NS
68: MIGRAN	0.4590	0.1448	NS	1.0568	0.3286	NS
69: MIGRAN	0.7246	0.2207	NS	1.6547	0.4987	NS
70: MRFITR	0.6382	0.2225	0.0338	1.1984	0.4461	0.0152
71: NAM	0.6883	0.0711	NS	1.5157	0.1569	NS
72: NAM	0.8481	0.0690	NS	1.8809	0.1532	NS
73: PARKIN	0.6129	0.0554	NS	1.3556	0.1222	NS
74: PERSH2	0.8420	0.0381	0.0004	1.9065	0.0854	0.0446
75: PETO	0.6262	0.1606	NS	1.4651	0.3737	NS
76: PEZZO2	1.3641	0.1251	0.0051	2.9784	0.2710	0.0118
77: PEZZOT	1.5483	0.1569	0.0005	3.4045	0.3415	0.0014
78: PRESCO	0.8289	0.1002	(0.0759)	1.9305	0.2318	NS
79: PRESCO	0.7383	0.0942	NS	1.6725	0.2122	NS
80: SEGI2	0.5679	0.0991	NS	1.2849	0.2208	NS
81: SEGI2	0.1503	0.1297	NS	0.3513	0.2865	NS
82: SHAW	1.1326	0.1121	NS	2.5298	0.2509	NS
83: SOBUE	0.4048	0.0594	NS	0.8893	0.1309	NS
84: SPEIZE	0.8772	0.0390	NS	1.9509	0.0870	NS
85: STOCKW	0.8822	0.0096	NS	1.9383	0.0210	NS
86: SVENSS	0.9255	0.1158	NS	2.0479	0.2573	NS
87: TENKAN	0.6211	0.1092	0.0001	1.4407	0.2484	0.0003
88: TSUGAN	0.1104	0.1169	NS	0.2461	0.2574	NS
89: TULINI	1.0019	0.0901	NS	2.2394	0.2008	NS
90: TULINI	1.2489	0.0862	(0.0728)	2.8447	0.1983	0.0098
91: TVERDA	0.6063	0.0773	NS	1.3657	0.1746	NS
92: TVERDA	0.9309	0.1838	NS	2.1244	0.4198	NS
93: WAKAI	0.6776	0.1099	(0.0831)	1.5073	0.2430	NS
94: WU	0.5916	0.1017	NS	1.3183	0.2263	NS
95: WYNDE6	0.9181	0.0373	NS	2.0237	0.0820	NS
96: WYNDE6	0.9796	0.0371	0.0102	2.1767	0.0825	0.0060
97: YAMAGU	0.6608	0.1223	NS	1.4756	0.2723	NS

[1]c = cigarettes/d; [2]Not significant (NS) indicates $P \geq 0.1$. P values in the range $0.05 \leq P < 0.1$ are shown in brackets.

Table 6 Amount smoked by current smokers - inverse-variance weighted simple regression analyses of β_1 based on best-fitting log + baseline model

Factor	Level	*n*	β_1 (95% CI)	*P*[1]	RR for 20 cigs/d
All		97	0.83 (0.80-0.86)		10.71
Sex	Male	55	0.79 (0.75-0.84)	< 0.05	9.50
	Female	34	0.82 (0.75-0.88)		10.21
	Combined	8	0.89 (0.84-0.94)		12.50
Study type	Case-control	49	0.86 (0.82-0.90)	< 0.1	11.48
	Prospective	48	0.80 (0.76-0.85)		9.85
Continent	North America	43	0.87 (0.84-0.89)	< 0.001	11.48
	Europe	32	0.83 (0.76-0.90)		10.65
	Asia	17	0.53 (0.45-0.61)		4.53
	Other	5	0.80 (0.61-0.99)		9.80
Publication year[2]	< 1990	40	0.83 (0.79-0.88)	< 0.1	10.71
	1990-1994	29	0.77 (0.70-0.84)		8.90
	1995-1999	28	0.87 (0.82-0.92)		11.94
Product[3]	Any product	20	0.75 (0.64-0.85)	NS	8.36
	Cigarettes +/-	61	0.85 (0.81-0.88)		11.09
	Cigarettes only	16	0.82 (0.74-0.90)		10.19
Unexposed	Never cigarettes	32	0.83 (0.76-0.90)	NS	10.49
	Never any product	65	0.84 (0.80-0.87)		10.76
Grouped midpoint age (yr)	< 50	14	0.79 (0.67-0.92)	< 0.1	9.57
	50-59	62	0.85 (0.82-0.89)		11.35
	60+	21	0.79 (0.71-0.84)		9.13
Cases in smokers	< 100	29	0.67 (0.56-0.78)	< 0.001	6.72
	100 to < 200	28	0.73 (0.64-0.83)		8.08
	200 to < 500	16	0.76 (0.65-0.87)		8.72
	500 to < 1000	16	0.80 (0.75-0.86)		9.87
	1000+	8	0.89 (0.85-0.92)		12.42

[1]Probability values for factor considered, presented as < 0.001, < 0.01, < 0.05, < 0.1 or not significant (NS) ($P \geq 0.1$); [2]Of principal publication for the study; [3]Any product = Smokes cigarettes and/or pipes and/or cigars; Cigarettes +/- = Smokes cigarettes with or without other products (pipes, cigars).

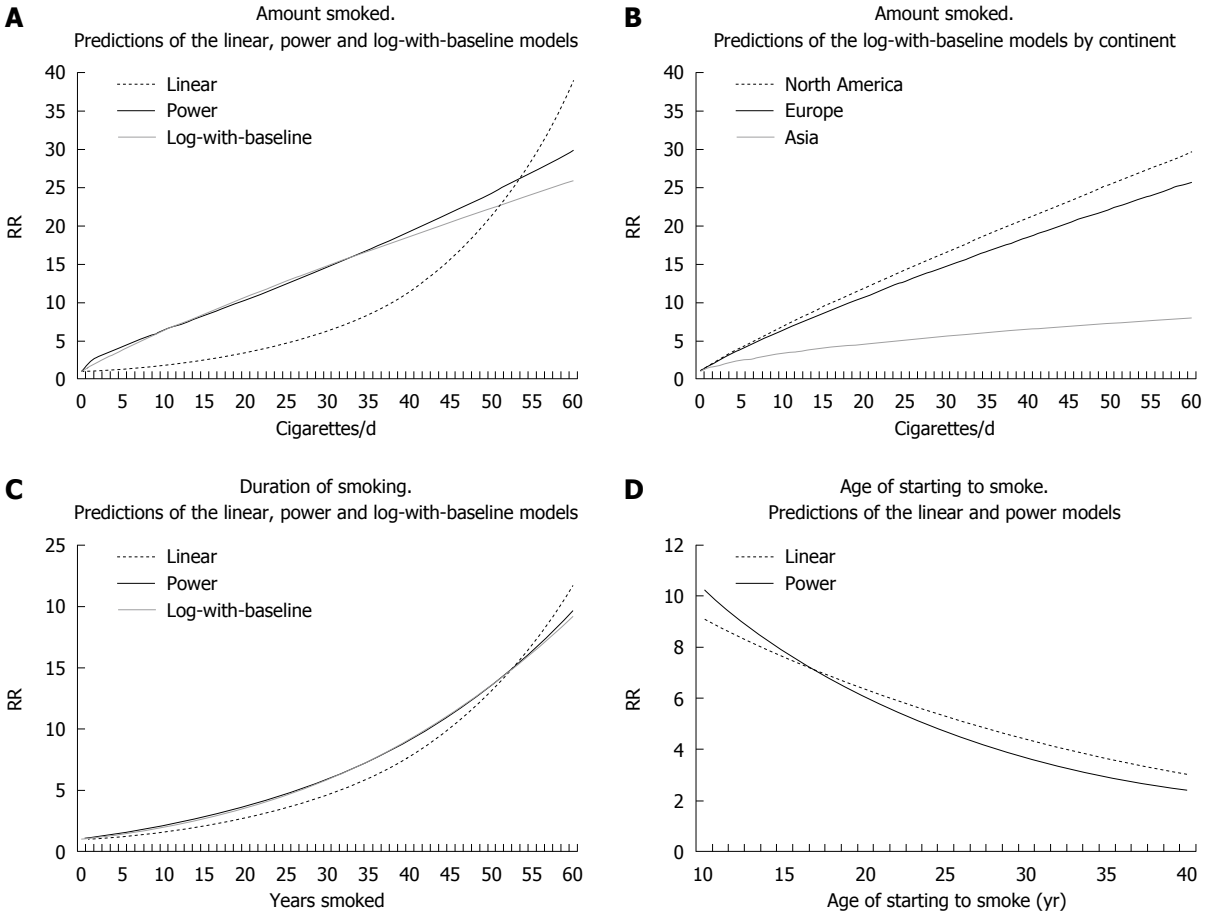

Figure 1 Model predictions. A: Amount smoked. Predictions of the linear, power and log-with-baseline models; B: Amount smoked. Predictions of the log-with-baseline models by continent; C: Duration of smoking. Predictions of the linear, power, and log-with-baseline models; D: Age of starting to smoke. Predictions of the linear and power models. The RR is plotted against number of cigarettes per day (A and B), years smoked (C) and age of starting to smoke (D). The linear model for amount smoked is the poorest fit to the data, other models shown fitting the data similarly well.

For the 97 blocks combined the RRs predicted by the log-with-baseline model for 5, 10, 20, 30, 40 and 50 cigs/d are, respectively, 3.86, 6.30, 10.71, 14.77, 18.62 and 22.31. The RRs are similar for the power model (4.30, 6.33, 10.34, 14.61, 19.24 and 24.29), but very different for the linear model (1.36, 1.84, 3.39, 6.25, 11.50 and 21.19). See also Figure 1A which compares model predictions, and Figure 1B which shows the predictions by continent.

Duration of smoking

Blocks[11] gives details of the 35 blocks, derived from 14 studies. CPS I and CPS Ⅱ provide 20 blocks, with data by sex and age. Three further studies provide sex-specific results, the remaining nine only providing one block each. Three studies are from Europe, two South America and one Asia, the remaining eight being from North America.

Table 7 summarizes the dose-response data. A clear increase in risk with increasing duration is evident, 34 blocks (97.1%) showing a greater RR for the longest than the shortest duration group, and 22 (62.9%) showing a strictly monotonic increase in RR.

Table 8 summarizes the analyses on model suitability. The exponential model is again very poor, explaining only 31.47% of the deviance. Other models differ little, explaining 88.49% to 89.96%. The best single parameter models are the best-fitting power model (89.96%) and log-with-baseline model (89.89%).

Fit Duration[11], gives full details for the further analyses using the linear model and best-fitting power and log-with-baseline models, laid out as Fit Amount Smoked[11].

Table 9 compares observed and fitted cases summed over blocks. Misfit is similar for all three models, and significant ($P < 0.001$), though its extent seems relatively moderate.

Table 10 gives fitted values of β_1 and SE and goodness-of-fit P values for the power and log-with-baseline models. We comment on six blocks where P is < 0.001 for both models, and three where P is < 0.001 for one model (Fit Duration[11] and Table 7 for further details). In three blocks (1: AMANDU, 29: HUMBLE, 35: PEZZO2) the RR associated with the lowest duration level is large, but the RRs associated with higher levels are not much larger (or even smaller). In another block (10: CPS I males age 65-74 years) the misfit comes from the lack of rise in risk over short duration levels, while in another (17: CPS Ⅱ males age 30-44 years) it is associated with the very high risk for the longest duration. In three other blocks (11: CPS I males age 75+ years, 15: CPS I females age 65-74 years, 20: CPS Ⅱ males age 65-74 years) the misfit is at least partly due to the non-monotonic dose-response.

Table 7 Duration of smoking by current smokers (yr) - dose-response data

Block: Study	Duration of smoking groupings (yr)	Mean values	RRs[1]
1: AMANDU	0-24, 25+	12.66, 41.28	5.92, 7.02 M
2: BEST	1-4, 5-9, 10-14, 15-19, 20-29, 30-39, 40+	2.64, 7.02, 12.03, 16.89, 24.03, 33.93, 51.12	1.60, 2.60, 2.30, 3.20, 4.10, 13.90, 14.20
3: BOUCOT	1-39, 40+	32.05, 51.14	42.40, 89.94 M
4: BUFFLE	1-30, 31-40, 41+	19.34, 35.56, 48.49	14.00, 14.70, 18.15 M
5: CEDERL	1-29, 30+	23.94, 41.09	1.80, 7.40 M
6: CEDERL	1-29, 30+	22.26, 39.47	1.60, 9.60 M
7: CPS I	1-29, 30+	23.70, 32.03	3.83, 6.56 M
8: CPS I	1-29, 30-34, 35-39, 40+	24.92, 31.91, 36.52, 43.07	5.58, 13.40, 16.93, 29.61 M
9: CPS I	1-29, 30-34, 35-39, 40-44, 45-49, 50+	23.57, 32.25, 37.75, 42.03, 46.80, 51.81	3.11, 6.57, 10.35, 15.21, 21.46, 33.11 M
10: CPS I	1-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60+	10.67, 30.00, 39.00, 42.21, 47.57, 52.05, 56.73, 62.87	5.46, 6.26, 5.86, 8.22, 12.48, 15.28, 18.86, 28.60
11: CPS I	1-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60+	20.00, 32.00, 37.00, 41.00, 46.25, 51.86, 57.00, 65.03	2.17, 2.27, 12.66, 3.47, 6.22, 6.31, 12.87, 13.23
12: CPS I	1-29, 30+	21.86, 31.37	6.16, 13.80 M
13: CPS I	1-29, 30-34, 35-39, 40+	23.29, 31.61, 36.72, 43.09	2.90, 6.91, 8.49, 19.48 M
14: CPS I	1-29, 30-34, 35-39, 40-44, 45-49, 50+	21.03, 31.92, 37.45, 42.21, 46.65, 52.80	1.51, 3.71, 4.73, 5.72, 7.78, 14.48 M
15: CPS I	1-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55+	21.69, 31.38, 37.19, 41.80, 47.14, 51.76, 57.56	2.30, 3.38, 3.67, 5.81, 7.01, 6.20, 3.32
16: CPS I	1-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55+	18.00, 30.00, 38.00, 41.75, 46.71, 52.25, 61.54	0.38, 3.11, 2.72, 3.22, 1.15, 1.85, 3.20
17: CPS II	1-29, 30+	19.15, 32.13	3.69, 108.27 M
18: CPS II	1-29, 30-34, 35-39, 40+	24.92, 31.91, 36.52, 43.07	7.72, 19.47, 25.01, 36.98 M
19: CPS II	1-29, 30-34, 35-39, 40-44, 45-49, 50+	23.57, 32.25, 37.75, 42.03, 46.80, 51.81	15.51, 17.86, 27.31, 44.71, 50.92, 72.07 M
20: CPS II	1-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60+	10.67, 30.00, 39.00, 42.21, 47.57, 52.05, 56.73, 62.87	9.97, 11.05, 20.45, 18.59, 24.33, 32.06, 40.43, 45.64
21: CPS II	1-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60+	20.00, 32.00, 37.00, 41.00, 46.25, 51.86, 57.00, 66.09	7.54, 4.53, 4.37, 16.30, 16.01, 13.40, 18.09, 21.79
22: CPS II	1-19, 20+	13.93, 24.23	10.98, 8.38
23: CPS II	1-29, 30-34, 35+	23.29, 31.61, 37.81	8.96, 18.34, 23.32 M
24: CPS II	1-29, 30-34, 35-39, 40-44, 45-49, 50+	21.03, 31.92, 37.45, 42.22, 46.65, 52.80	6.50, 13.04, 17.07, 20.56, 23.94, 28.45 M
25: CPS II	1-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55+	21.69, 31.38, 37.19, 41.80, 47.14, 51.76, 57.56	6.64, 5.57, 10.19, 12.96, 15.79, 18.75, 19.34
26: CPS II	1-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60+	18.00, 30.00, 38.00, 42.20, 46.71, 52.23, 57.10, 65.06	3.36, 7.92, 6.31, 8.14, 11.68, 9.45, 9.18, 19.88
27: DEAN2	1-19, 20+	15.09, 38.28	3.21, 3.84 M
28: DEAN2	1-19, 20+	13.93, 35.85	0.98, 5.88
29: HUMBLE	1-29, 30-39, 40-49, 50+	17.18, 34.07, 44.43, 56.60	15.45, 17.54, 19.61, 17.27
30: KAISE2	1-39, 40+	23.76, 51.12	4.86, 15.64 M
31: KAISE2	1-39, 40+	22.73, 48.96	9.09, 30.41 M
32: KATSOU	1-29, 30+	14.58, 42.43	1.29, 7.43 M
33: LIAW	1-20, 21-30, 31+	14.32, 26.18, 44.79	0.90, 2.60, 4.70
34: MATOS	1-24, 25-39, 40+	12.75, 31.16, 50.57	5.20, 7.40, 10.20 M
35: PEZZO2	1-35, 36+	17.15, 48.89	16.25, 26.77 M

[1]M indicates a strictly monotonic rise in RR with increasing duration of smoking.

In the remaining block (9: CPS I males age 55-64 years) the rise is monotonic, but the relatively small RR for the shortest duration does not fit in well with the large RRs for longer durations.

Table 11 presents results of weighted simple regression analysis of β_1 for the power model. Significance at $P < 0.05$ is only seen for midpoint age, with higher β_1 values for lower ages. In forward stepwise regressions (not shown), the model included in succession midpoint age ($P < 0.05$), number of cases in smokers ($P < 0.05$), smoking product ($P < 0.05$) and unexposed group ($P < 0.01$). The final model associated increased risk with younger age, larger numbers of cases, smoking of cigarettes only or cigarettes ± other products (compared to smoking any product) and with the unexposed being never any product (rather than never cigarettes).

For the 35 blocks combined the RRs predicted by the power model for durations of 10, 20, 30, 40 and 50 years are, respectively, 2.21, 3.75, 5.96, 9.11 and 13.54. Figure 1C compares model predictions.

Age of starting to smoke

Blocks[11] gives details of the 27 blocks, deriving from 15 studies. One study gives results by age and sex, two by age for males, and five by sex but not age, the remaining seven studies only providing one block each. There are similar numbers of blocks from North America (9), Europe (9) and Asia (8), only one being from elsewhere.

Table 12 summarizes the dose-response data. A relationship of risk to age of starting is not consistently seen. While 13 blocks (48.1%) show a strictly monotonic decline in risk as starting age increases, with risk often substantially higher in early starters, and 6 (22.2%) blocks show a similar non-monotonic tendency, 8 (29.6%) blocks (1, 2, 7-9, 16, 20, 21) show no such tendency.

Table 13 summarizes the analyses on model suitability,

Table 8 Comparing the suitability of different models relating log RR to duration of smoking by current smokers, expressed as d = years smoked/10

Model	Parameter value[1]	Fitted coefficient(s) (SE)	Deviance	DF	Deviance explained (%)
Null	-	-	6161.11	35	
Linear: log RR = $\beta_1 d$	-	β_1 = 0.5134 (0.0069)	672.01	34	89.09
Quadratic: log RR = $\beta_1 d + \beta_2 d^2$	-	β_1 = 0.6718 (0.0237), β_2 = -0.0300 (0.0043)	623.20	33	89.88
Cubic: log RR = $\beta_1 d + \beta_2 d^2 + \beta_3 d^3$	-	β_1 = 0.6788 (0.0607), β_2 = -0.0330 (0.0243), β_3 = 0.0003 (0.0025)	623.19	32	89.89
Power: log RR = $\beta_1 d^Y$	Y = 0.50	β_1 = 1.1576 (0.0156)	682.56	34	
	Y = 0.72	β_1 = 0.8180 (0.0110)	619.30	34	
	Y = 0.73	β_1 = 0.8049 (0.0108)	618.93	34	
	Y = 0.74	β_1 = 0.7919 (0.0106)	618.75	34	89.96
	Y = 0.75	β_1 = 0.7791 (0.0105)	618.76	34	
	Y = 0.76	β_1 = 0.7665 (0.0103)	618.95	34	
	Y = 1.00	β_1 = 0.5134 (0.0069)	672.01	34	
	Y = 2.00	β_1 = 0.0863 (0.0013)	1426.44	34	
Log: log RR = $\beta_1 \log d$	-	β_1 = 1.5870 (0.0215)	708.96	35	88.49
Exponential: log RR = $\beta_1 \exp d$		β_1 = 0.0045 (0.0001)	4222.01	35	31.47
Log-with-baseline: log RR = $\beta_1 \log (1 + Wd)$	W = 0.10	β_1 = 6.4054 (0.0861)	629.58	34	
	W = 0.15	β_1 = 4.6503 (0.0625)	623.99	34	
	W = 0.18	β_1 = 4.0572 (0.0545)	622.82	34	
	W = 0.19	β_1 = 3.9001 (0.0524)	622.69	34	
	W = 0.20	β_1 = 3.7581 (0.0505)	622.67	34	89.89
	W = 0.21	β_1 = 3.6293 (0.0488)	622.74	34	
	W = 0.22	β_1 = 3.5117 (0.0472)	622.89	34	

[1]Note that we only sought the best-fitting value of W and Y to two decimal places.

Table 9 Duration of smoking by current smokers - observed and fitted lung cancers for the linear, best power and best log-with-baseline model, with β_1 fitted separately for each block

Midpoint years smoked	Observed[1]	Fitted[2]		
		Linear model	Best power model	Best log-with-baseline model
< 15	109.36	89.10	93.61	91.19
15 to < 30	605.64	584.79	666.12	645.42
30 to < 45	3968.47	3917.11	4000.71	4012.22
45+	3493.56	3573.95	3499.85	3499.95
Never smoked	2533.97	2546.03	2450.69	2462.20
Total	10710.98	10710.98	10710.98	10710.98
Fit statistic[3]		28.14	25.70	24.53

[1]Observed pseudo-number of lung cancer cases, summed over blocks; [2]Fitted pseudo-number of lung cancer cases, summed over blocks. For each study, the fitted number of cases for each block is calculated from the numbers at risk and the fitted RRs by years smoked, derived from the fitted value of β_1; [3]Based on summation of (observed-fitted)^2/fitted, the summation also including terms for the observed and fitted total numbers of controls (not shown). The statistic can be considered to be approximately chisquared distributed on 6 df, and is significant at $P < 0.001$ for all three models.

taking (70-age at starting) as a duration-like measure. The exponential model again is the poorest, explaining only 60.24% of the deviance, and the log model is also poorer than the other models. Although slightly more deviance is explained by the cubic model, the best-fitting power model is the best simple model, explaining 88.29% of the deviance. Log-with-baseline models were also tried (not shown), but the best-fitting value of W was extremely low, so it became essentially identical to the linear model, having the same deviance.

Fit Age Start[11], gives full details of the further analyses using the linear and best-fitting power models.

Table 14 compares observed and fitted cases summed over blocks. Both the linear and best-fitted power models fit well.

Table 15 gives fitted values of β_1 and SE and goodness-of-fit *P* values. For no block does either model show misfit at $P < 0.01$, though misfits at $P < 0.05$ are sometimes seen. We comment on four blocks with some evidence ($P < 0.1$) of misfit to both models (Table 12 and Fit Age Start[11] for further details). For block 4 (CPS I males aged 55-69) the misfit seems due to the relatively small decline in risk between age of start 1-14 and 15-19 years, compared to a greater decline subsequently. For block 5 (CPS I males aged 70-84 years), risk again declines substantially over ages of start 15-19, 20-24 and 25+ years, but risk is slightly less at age 1-14 than 15-19 years. For block 12 (ENGELA males), risk decreases from age 1-19 to 20-29 years but then falls no further. For block 17 (LIAW), the pattern is non-monotonic.

Table 16 presents results of the weighted simple regression analyses of β_1 for the power model. Various fac-

Table 10 Duration of smoking by current smokers - fitted values for of β1 and SE, and *P* values for goodness-of-fit tests for the best-fitting power model and log-with-baseline model

Block: Study	Log-with-baseline model[1] log RR = β1 [1 + (0.2 yr/10)]			Power model[1] log RR = β1 (yr/10)$^{0.74}$		
	β1	SE β1	*P* (fit)[2]	β1	SE β1	*P* (fit)[2]
1: AMANDU	1.5114	0.5504	0.0030	0.3454	0.1210	0.0046
2: BEST	3.7163	0.4210	(0.0548)	0.7995	0.0908	0.0469
3: BOUCOT	6.7731	1.6574	NS	1.4235	0.3482	NS
4: BUFFLE	4.5141	0.4888	0.0466	0.9570	0.1029	(0.0780)
5: CEDERL	3.4044	0.7166	NS	0.7147	0.1512	NS
6: CEDERL	3.3566	0.7692	NS	0.6990	0.1609	NS
7: CPS I	3.6386	1.4531	NS	0.7530	0.3009	NS
8: CPS I	5.7248	0.4753	(0.0947)	1.2122	0.1011	(0.0518)
9: CPS I	4.8058	0.2032	0.0003	1.0130	0.0429	0.0002
10: CPS I	3.7681	0.1557	0.0002	0.7976	0.0328	0.0010
11: CPS I	3.1437	0.1768	0.0079	0.6566	0.0369	0.0073
12: CPS I	5.1328	1.5211	NS	1.0518	0.3119	NS
13: CPS I	3.9960	0.3377	0.0308	0.8342	0.0708	0.0165
14: CPS I	2.8575	0.1844	(0.0637)	0.5999	0.0388	0.0383
15: CPS I	2.6384	0.1872	0.0000	0.5544	0.0394	0.0000
16: CPS I	1.5443	0.3346	NS	0.3238	0.0702	NS
17: CPS II	9.5482	2.4662	0.0149	1.9485	0.5160	0.0076
18: CPS II	6.1160	0.5675	NS	1.2874	0.1199	NS
19: CPS II	5.9905	0.3222	(0.0749)	1.2722	0.0684	(0.0921)
20: CPS II	4.5913	0.1996	0.0058	0.9714	0.0421	0.0210
21: CPS II	3.6604	0.2069	NS	0.7645	0.0432	NS
22: CPS II	5.1073	3.0925	NS	1.1011	0.6362	NS
23: CPS II	5.8103	0.4709	NS	1.2180	0.0988	NS
24: CPS II	4.9498	0.2256	NS	1.0461	0.0476	NS
25: CPS II	4.1028	0.1646	NS	0.8656	0.0347	NS
26: CPS II	3.3310	0.1915	NS	0.6993	0.0402	NS
27: DEAN2	2.1038	0.3399	NS	0.4515	0.0723	NS
28: DEAN2	2.9155	0.6412	NS	0.5984	0.1334	NS
29: HUMBLE	4.2780	0.2807	0.0091	0.9005	0.0590	0.0148
30: KAISE2	3.8758	0.4357	NS	0.8202	0.0922	NS
31: KAISE2	4.7883	0.5320	NS	1.0198	0.1130	NS
32: KATSOU	2.9305	0.7339	NS	0.6031	0.1527	NS
33: LIAW	2.4277	0.4372	NS	0.5093	0.0921	NS
34: MATOS	3.4356	0.5336	NS	0.7276	0.1124	NS
35: PEZZO2	3.3022	0.5283	0.0002	0.7260	0.1137	0.0005

[1]Years smoked; [2]Not significant (NS) indicates $P \geq 0.1$. *P* values in the range $0.05 \leq P < 0.1$ are shown in brackets.

tors are significant at $P < 0.05$, including number of lung cancer cases ($P < 0.001$), continent ($P < 0.01$), publication year ($P < 0.01$), sex ($P < 0.05$) and product smoked ($P < 0.05$), though in a forward stepwise model (details not shown), only two factors were included: number of lung cancer cases ($P < 0.001$) and midpoint age ($P < 0.05$). However, the relationship of β1 to number of cases was not smooth (higher risks for 100 to < 200, and 500 to < 1000 cases and lower risks for < 100, 200 to < 500 and 1000+) so the result is difficult to interpret. The association with age is related to a lower β1 in older subjects (aged 60+ years).

For the 31 blocks combined, the RRs predicted for age of start 12.5, 15, 17.5, 20, 25 and 30 years are, respectively, 8.94, 7.80, 6.83, 5.99, 4.66 and 3.66 for the power model and 8.31, 7.57, 6.91, 6.30, 5.24 and 4.36 for the linear model (Figure 1D).

DISCUSSION

In our earlier review[1] of the evidence relating smoking to lung cancer, we demonstrated a clear dose-response with the three measures considered, risk increasing with increasing amount smoked and duration and with decreasing age of starting. We extend this work by fitting parametric models to the dose-relationships.

We tried various models. The most useful were the "linear model" (log RR = $\beta_1 d$), the "power model" (log RR = $\beta_1 d^Y$) and the "log-with-baseline model" [log RR = $\beta_1 \log (1 + Wd)$], where d is dose. For amount smoked, the linear model proved inadequate, but a reasonable fit was found with the other two models, the best fit being for the log-with-baseline model with W = 0.81. For duration, all three models were reasonable, the best being the power model with Y = 0.74. For age of starting, where we used a duration-like dose measure based on (70 - age of starting to smoke), the best-fitting model was again the power model, here with Y = 1.44.

Inverse-variance weighted analyses were also carried out to identify sources of heterogeneity in β1. For amount smoked, as expected from our earlier review[1], the major source was continent, the fitted slope being much less

Table 11 Duration of smoking by current smokers - inverse-variance weighted simple regression analyses of β1 based on best-fitting power model

Factor	Level	*n*	β1 (95%CI)	*P*[1]	RR for 30 yr smoked
All		35	0.79 (0.72-0.86)		5.94
Sex	Male	18	0.84 (0.73-0.94)	NS	6.59
	Female	15	0.74 (0.64-0.85)		5.35
	Combined	2	0.79 (0.45-1.12)		5.89
Study type	Case-control	7	0.73 (0.50-0.97)	NS	5.24
	Prospective	28	0.80 (0.72-0.87)		6.02
Continent	North America	27	0.81 (0.73-0.88)	NS	6.19
	Europe	5	0.55 (0.20-0.90)		3.44
	Asia	1	0.51 (-0.11-1.13)		3.15
	Other	2	0.73 (0.19-1.27)		5.15
Publication year[2]	< 1990	8	0.76 (0.52-0.99)	NS	5.50
	1990-1994	2	0.44 (-0.20-1.09)		2.73
	1995-1999	25	0.80 (0.72-0.88)		6.08
Product[3]	Any product	4	0.50 (0.18-0.83)	NS	3.12
	Cigarettes +/-	10	0.86 (0.73-0.98)		6.89
	Cigarettes only	21	0.78 (0.70-0.87)		5.85
Unexposed	Never cigarettes	19	0.77 (0.69-0.86)	NS	5.71
	Never any product	16	0.85 (0.71-0.99)		6.75
Grouped midpoint age (yr)	< 50	8	1.06 (0.78-1.34)	< 0.05	11.00
	50-59	13	0.87 (0.75-0.99)		7.04
	60+	14	0.73 (0.65-0.82)		5.21
Cases in smokers	< 100	14	0.62 (0.39-0.84)	NS	4.01
	100 to < 200	9	0.73 (0.59-0.87)		5.19
	200 to < 500	6	0.78 (0.67-0.89)		5.18
	500 to < 1000	5	0.95 (0.80-1.10)		8.58
	1000+	1	0.80 (0.59-1.01)		6.04

[1]Probability values for factor considered, presented as < 0.001, < 0.01, < 0.05, < 0.1 or not significant (NS) ($P \geqslant 0.1$); [2]Of principal publication for the study; [3]Any product = Smokes cigarettes and/or pipes and/or cigars; Cigarettes +/- = Smokes cigarettes with or without other products (pipes, cigars).

Table 12 Age of starting to smoke by current smokers (yr) - dose-response data

Block: Study	Age of starting to smoke groupings (yr)	Mean values	RRs[1]
1: CEDERL	< 17, 17-18, 19+	13.83, 17.52, 22.45	6.40, 9.80, 6.50
2: CEDERL	< 17, 17-18, 19+	14.31, 17.56, 24.32	0.61, 1.84, 1.99
3: CPS I	< 15, 15-19, 20-24, 25+	11.98, 16.74, 21.29, 28.47	15.00, 9.71, 7.14, 3.43 M
4: CPS I	< 15, 15-19, 20-24, 25+	11.59, 16.56, 21.00, 30.90	18.16, 16.32, 12.00, 5.21 M
5: CPS I	< 15, 15-19, 20-24, 25+	11.19, 16.61, 21.07, 35.00	14.03, 16.60, 8.66, 1.71
6: CPS I	< 15, 15-19, 20-24, 25+	12.71, 16.88, 21.26, 31.51	9.00, 5.00, 4.00, 1.50 M
7: CPS I	< 20, 20-24, 25+	16.02, 20.95, 33.29	2.59, 3.23, 2.62
8: DEAN3	< 15, 15-19, 20-24, 25+	11.73, 16.67, 21.19, 29.95	5.67, 7.01, 6.86, 6.80
9: DEAN3	< 15, 15-19, 20-24, 25+	12.45, 16.95, 21.15, 32.19	2.41, 2.90, 2.95, 3.70
10: DORN	< 15, 15-19, 20-24, 25+	11.63, 16.43, 21.23, 30.88	23.42, 16.25, 11.06, 5.18 M
11: DORN	< 15, 15-19, 20-24, 25+	11.37, 16.81, 20.69, 32.63	14.18, 11.31, 7.94, 4.95 M
12: ENGELA	< 20, 20-29, 30+	15.08, 22.23, 35.10	7.42, 3.60, 3.79
13: ENGELA	< 20, 20-29, 30+	15.80, 22.63, 35,80	11.29, 8.15, 2.73 M
14: GAO2	< 20, 20-29, 30+	15.11, 22.17, 35.94	8.62, 6.44, 2.15 M
15: HIRAYA	< 20, 20+	14.90, 24.02	5.71, 4.35 M
16: HIRAYA	< 20, 20+	15.87, 27.40	0.78, 2.46
17: LIAW	< 21, 21-24, 25+	15.70, 21.94, 32.03	4.60, 5.90, 1.50
18: MATOS	< 15, 15-19, 20+	11.96, 16.64, 23.49	11.30, 8.60, 5.30 M
19: MIGRAN	< 16, 16-19, 20+	12.60, 17.19, 23.71	10.03, 6.93, 7.79
20: MIGRAN	< 16, 16-19, 20+	12.94, 17.31, 26.17	7.17, 8.29, 7.98
21: MRFITR	< 16, 16-17, 18-19, 20-21, 22-23, 24+	12.93, 16.45, 18.31, 20.40, 22.35, 27.74	45.91, 67.17, 50.54, 27.09, 60.06, 23.91
22: SEGI2	< 20, 20-22, 23+	15.28, 20.77, 26.60	8.21, 5.56, 1.83 M
23: SEGI2	< 20, 20-22, 23+	14.87, 20.90, 28.96	8.70, 5.68, 3.56 M
24: SEGI2	< 20, 20-22, 23+	14.37, 20.59, 30.87	3.26, 1.70, 1.52 M
25: SVENSS	< 19, 19-25, 26+	15.04, 21.31, 33.89	7.82, 13.08, 5.61
26: WAKAI	< 20, 20-29, 30+	14.91, 22.14, 37.23	3.69, 4.62, 2.08
27: WU	< 19, 19-24, 25+	15.06, 20.71, 31.44	10.32, 3.57, 1.55 M

[1]M indicates a strictly monotonic decline in RR with increasing age of starting to smoke.

Table 13 Comparing the suitability of different models relating log *RR* to age of starting smoke by current smokers, expressed as d = (70-age at start)/10

Model	Parameter value[1]	Fitted coefficient(s) (SE)	Deviance	DF	Deviance explained (%)
Null	-	-	2145.30	27	
Linear: log RR = $\beta_1 d$	-	β_1 = 0.3681 (0.0085)	276.67	26	87.10
Quadratic: log RR = $\beta_1 d + \beta_2 d^2$	-	β_1 = 0.1987 (0.0349) β_2 = 0.0318 (0.0064)	251.63	25	88.27
Cubic: log RR = $\beta_1 d + \beta_2 d^2 + \beta_3 d^3$	-	β_1 = -0.0415 (0.2143) β_2 = 0.1304 (0.0870) β_3 = -0.0100 (0.0088)	250.34	24	88.33
Power: log RR = $\beta_1 d^Y$	Y = 0.75	β_1 = 0.5515 (0.0129)	316.69	26	
	Y = 1.00	β_1 = 0.3681 (0.0085)	276.67	26	
	Y = 1.42	β_1 = 0.1825 (0.0042)	251.27	26	
	Y = 1.43	β_1 = 0.1794 (0.0041)	251.24	26	
	Y = 1.44	β_1 = 0.1764 (0.0041)	251.23	26	88.29
	Y = 1.45	β_1 = 0.1734 (0.0040)	251.25	26	
	Y = 1.46	β_1 = 0.1705 (0.0039)	251.28	26	
	Y = 1.50	β_1 = 0.1592 (0.0037)	251.67	26	
	Y = 2.00	β_1 = 0.0668 (0.0015)	284.04	26	
Log: log RR = $\beta_1 \log d$	-	β_1 = 1.1460 (0.0270)	340.23	26	84.14
Exponential: log RR = $\beta_1 \exp d$	-	β_1 = 0.0058 (0.0002)	852.85	26	60.24

[1]Note that we only sought the best-fitting value of Y to two decimal places.

Table 14 Age of starting to smoke by current smokers - observed and fitted lung cancers for the linear and best power model, with β_1 fitted separately for each block

Age of starting (yr)	Observed[1]	Fitted[2]	
		Linear model	Best power model
< 16	1304.92	1294.48	1387.73
16 to < 20	1964.76	1906.55	1921.20
20 to < 24	1227.48	1266.99	1216.48
25+	2173.90	2219.26	2122.14
Never smoked	894.41	878.20	917.92
Total	7565.47	7565.47	7565.47
Fit statistic[3]		4.77	9.75

[1]Observed pseudo-number of lung cancer cases, summed over blocks; [2]Fitted pseudo-number of lung cancer cases, summed over blocks. For each study, the fitted number of cases for each block is calculated from the numbers at risk and the fitted relative risks for age of starting to smoke, derived from the fitted value of β_1; [3]Based on summation of (observed-fitted)^2/fitted, the summation also including terms for the observed and fitted total numbers of controls (not shown). The statistic can be considered to be approximately chisquared distributed on 6 df and is not significant ($P > 0.1$) for both models.

steep for Asian than European or North American studies. However, it proved more difficult to identify meaningful major sources for the other measures.

We discuss various issues relating to interpretation of these findings.

Adequacy of literature search and publication bias

All the data used came from the IESLC database. The source paper[1] demonstrated that the search was comprehensive, though limited to papers published before 2000 and studies of 100+ cases. Publication bias was discussed earlier[1], evidence for its existence being considered not strong. The probability of dose-response results being published might depend on the strength of the overall relationship seen. While clearly demonstrated for passive smoking and lung cancer[3], this seems less relevant here, the association with active smoking being so strong. Nevertheless, some publication bias may exist.

There are various reasons why the fitted dose-relationships may not accurately reflect the true relationships.

Misclassification of smoking status

It is well-documented (*e.g.*,[12,13]) that some subjects deny current or past smoking, so increasing the apparent lung cancer risk in reported never smokers and biasing downwards the estimated smoking RR. Such misclassification is difficult to adjust for, as it varies by aspects of study design, the questions asked, and also by sex, age, location and other demographics. Indeed, higher denial rates in Asian populations[14] may contribute to the markedly weaker observed associations seen in Asia.

In prospective studies there is an additional problem, especially in studies with long-term follow-up with no re-interviews to update smoking status. In particular, some subjects classified at baseline as current smokers may quit during follow-up. Also some never smokers may start, though this is less likely given the subjects' age at baseline in many studies.

Misclassification of amount smoked

Similar problems arise. Subjects may understate (or overstate) the amount they smoke, and during follow-up in prospective studies, may reduce or increase the amount smoked. Although some studies, particularly case-control, may ask questions on habits at various times during the subject's smoking career, the data reported may relate to average consumption. Someone smoking, say, 30 cigarettes/d for 20 years, then 10 cigarettes/d for 20 years, may not have the same risk as someone smoking 20 cigarettes/d for the whole 40 years period. Difficulties in re-

Table 15 Age of starting to smoke by current smokers - fitted values of β_1 and SE, and *P* values for goodness-of-fit tests for the linear model and for the best-fitting power model

Block: Study	Linear model[1] log RR = β_1(70 - a)/10			Power model[1] log RR = β_1(70 - a)/$10^{1.44}$		
	β_1	SE β_1	*P* (fit)[2]	β_1	SE β_1	*P* (fit)[2]
1: CEDERL	0.0385	0.0082	NS	0.1828	0.0394	NS
2: CEDERL	0.0140	0.0089	NS	0.0699	0.0445	NS
3: CPS I	0.0461	0.0041	0.0490	0.2167	0.0188	NS
4: CPS I	0.0524	0.0029	(0.0959)	0.2399	0.0135	(0.0579)
5: CPS I	0.0512	0.0047	0.0195	0.2408	0.0223	0.0307
6: CPS I	0.0301	0.0036	(0.0510)	0.1480	0.0175	NS
7: CPS I	0.0237	0.0046	NS	0.1168	0.0238	0.0828
8: DEAN3	0.0332	0.0041	NS	0.1482	0.0189	0.0342
9: DEAN3	0.0209	0.0039	NS	0.0960	0.0187	NS
10: DORN	0.0549	0.0037	(0.0874)	0.2527	0.0166	NS
11: DORN	0.0455	0.0027	NS	0.2086	0.0125	NS
12: ENGELA	0.0360	0.0037	0.0109	0.1682	0.0171	0.0269
13: ENGELA	0.0442	0.0045	NS	0.2186	0.0221	NS
14: GAO2	0.0394	0.0066	NS	0.1909	0.0318	NS
15: HIRAYA	0.0317	0.0022	NS	0.1497	0.0104	0.0288
16: HIRAYA	0.0207	0.0029	NS	0.1086	0.0152	NS
17: LIAW	0.0296	0.0050	0.0396	0.1448	0.0242	(0.0661)
18: MATOS	0.0411	0.0063	NS	0.1933	0.0296	NS
19: MIGRAN	0.0373	0.0087	NS	0.1612	0.0385	NS
20: MIGRAN	0.0400	0.0108	NS	0.1859	0.0519	NS
21: MRFITR	0.0686	0.0237	NS	0.2931	0.1055	NS
22: SEGI2	0.0468	0.0140	NS	0.2325	0.0639	NS
23: SEGI2	0.0440	0.0128	NS	0.1974	0.0557	NS
24: SEGI2	0.0192	0.0109	NS	0.0945	0.0500	NS
25: SVENSS	0.0432	0.0051	NS	0.2064	0.0246	NS
26: WAKAI	0.0275	0.0068	NS	0.1244	0.0318	NS
27: WU	0.0348	0.0063	NS	0.1741	0.0305	NS

[1]a = Age of starting to smoke; [2]Not significant (NS) indicates $P \geqslant 0.1$. *P* values in the range $0.05 \leqslant P < 0.1$ are shown in brackets.

Table 16 Age of starting to smoke by current smokers - inverse-variance weighted simple regression analyses of β_1 based on best-fitting power model

Factor	Level	*n*	β_1 (95%CI)	*P*[1]	RR for 15 yr start
All		27	0.18 (0.16-0.20)		7.80
Sex	Male	17	0.19 (0.17-0.21)	< 0.05	9.49
	Female	9	0.14 (0.11-0.17)		5.14
	Combined	1	0.14 (0.04-0.25)		5.40
Study type	Case-control	10	0.15 (0.11-0.20)	NS	5.94
	Prospective	17	0.18 (0.16-0.20)		8.37
Continent	North America	9	0.21 (0.19-0.23)	< 0.01	11.49
	Europe	9	0.16 (0.13-0.19)		6.38
	Asia	8	0.14 (0.11-0.17)		5.17
	Other	1	0.19 (0.08-0.31)		9.50
Publication year[2]	< 1990	11	0.15 (0.11-0.19)	< 0.01	5.59
	1990-1994	4	0.14 (0.11-0.17)		5.17
	1995-1999	12	0.20 (0.18-0.22)		10.37
Product[3]	Any product	3	0.17 (0.11-0.24)	< 0.05	7.65
	Cigarettes +/-	16	0.19 (0.17-0.21)		9.09
	Cigarettes only	8	0.13 (0.09-0.17)		4.69
Unexposed	Never cigarettes	4	0.19 (0.13-0.25)	NS	9.05
	Never any product	23	0.17 (0.15-0.20)		7.66
Grouped midpoint age (yr)	< 50	7	0.18 (0.13-0.23)	NS	7.73
	50-59	9	0.21 (0.17-0.25)		10.98
	60+	11	0.17 (0.14-0.19)		6.88
Cases in smokers	< 100	12	0.14 (0.11-0.17)	< 0.001	5.09
	100 to < 200	7	0.20 (0.16-0.24)		9.88
	200 to < 500	4	0.17 (0.23-0.20)		7.02
	500 to < 1000	3	0.23 (0.20-0.26)		14.57
	1000+	1	0.15 (0.11-0.19)		5.71

[1]Probability values for factor considered, presented as < 0.001, < 0.01, < 0.05, < 0.1 or not significant (NS) ($P \geqslant 0.1$); [2]Of principal publication for the study; [3]Any product = Smokes cigarettes and/or pipes and/or cigars; Cigarettes +/- = Smokes cigarettes with or without other products (pipes, cigars).

membering smoking history also form part of the problem. Also the dose of smoke constituents received may not be directly proportional to the amount smoked[15].

Misclassification of duration and age of starting

Subjects may not remember the exact age of starting, and indeed there may be differences in definition between studies - age of first trying a cigarette, or age of starting to smoke regularly? Also duration may not represent a continuous period. Risk may be affected by intermediate quit periods, which may be asked about differently in different studies.

Estimating midpoints of ranges

The statistical methods used require estimates of midpoints of ranges used. We have not attempted sensitivity analyses based on alternative procedures for defining midpoints.

Use of pseudo-numbers

Our methodology requires knowledge, for each block, of the numbers of cases and controls (or at risk) in each smoking group. As such data are not always provided, and indeed for covariate-adjusted data are only hypothetical, we used the method of Hamling *et al*[4] to estimate pseudo-numbers corresponding exactly to the reported RRs and CIs. These pseudo-numbers have been shown[16] to allow accurate estimation of RRs and CIs relative to a different base group from that used originally, and should be adequate for model fitting. This issue seems less important than others considered so far.

Adjustment for other smoking variables

Our analyses compare risk relative to never smokers, all the RRs in any block being adjusted for the same variables. As RRs relative to never smokers cannot be adjusted for other smoking variables, we necessarily restricted attention to estimates adjusted for age and non-smoking characteristics. This is possibly unfortunate as, for example, later starters may smoke less than earlier starters. In theory one could study the extent of such bias based on studies presenting risk (compared to never smokers) jointly by more than one dose measure. However, few studies present such data and we did not investigate this.

Use of simple models based on published results

We restricted attention to models of a relatively simple functional form, partly as it is much easier to explain results and conduct tests of heterogeneity where differences between blocks can be expressed in terms of one parameter (β_1). Also, the numerous data uncertainties may not justify a more complex approach. Such an approach is better pursued using individual person data from large studies. This would allow fitting of models simultaneously accounting for amount smoked and duration, and allow a more precise risk estimation. In the context of a systematic review and meta-analysis, involving many studies conducted years ago with the data unlikely to be accessible, we made no attempt to obtain individual data sets.

Model fit

Goodness-of-fit has been studied in various ways. First, we used the "pool-first" approach[9,10] to compare the deviance of models with a common β_1 per block but a different functional form of the dose-relationship. The exponential (log RR = $\beta_1$exp d) and the log model (log RR = $\beta_1$log d) clearly fitted substantially worse than other models, and were not pursued further. Also, the power model (log RR = $\beta_1 d^Y$) and the log-with-baseline model [log RR = $\beta_1$log (1 + Wd)] generally fitted better than the linear model (log RR = β_1d), though for age of starting the best-fitting log-with-baseline model had such a low estimate of W that it became equivalent to the linear model. While the deviance of the linear model was reduced by adding quadratic and cubic terms this advantage was small. We concentrated most on the power and/or log-with-baseline models, given their greater simplicity, and the fact that the cubic model fitted worse than these alternatives for amount smoked and not materially better for duration or age of start.

We then restricted attention to the linear, power and, except for age of start, the log-with-baseline model, fitting separate β_1 values to each block. We investigated goodness-of-fit by studying plots of observed and predicted RRs (not shown), and by comparing observed and predicted numbers, both within block (Fit Amount Smoked[11], Fit Duration, and Fit Age Start[11]) and summed over block (Tables 4, 9 and 14). This allowed two general conclusions. First, the best models (log-with-baseline for amount smoked, power for duration and age of start) fitted the shape of the dose relationship well. Given the large number of cases analyzed (54245 for amount smoked, 10711 for duration and 7575 for age of start) it is unsurprising that formal misfit existed for amount smoked and duration, but this seems relatively unimportant. Second, there were significant misfits for some blocks. The results section comments on the worst cases. Sometimes these are due to unusual response patterns, difficult to fit by any plausible model, sometimes to differing response patterns in different blocks. Thus, for amount smoked, there are some blocks where the slope flattens off at high consumption, but others where the reverse is true. The explaination for this is unclear, but attempting to account for it by more complex models seems unattractive, as compared to the models selected, which involve a common shape and variation only in slope (β_1).

Sources of heterogeneity

We carried out weighted regression analyses to investigate sources of heterogeneity. While some factors (*e.g.*, age and sex) could be better evaluated using pooled analyses based on individual person data, and problems arise from correlations between variables studied, these analyses should detect major sources.

For amount smoked, these analyses only identified

continent as a significant factor, other associations seen in the simple analyses becoming non-significant once continent was accounted for. The smaller β_1 for Asian studies is consistent with our earlier analyses[16], and may relate to higher denial rates of smoking in Asia.

For duration and age of starting, the regression analyses showed a tendency for β_1 to be greater in studies involving more lung cancer cases and studies of younger people. Higher values in males than females and lower values in Asian studies were not independently significant. There was also some evidence for duration of higher β_1 values for smoking cigarettes, than smoking any product.

Comparison with some previous work

Attempts have been made before to model the relationship of lung cancer to amount smoked and duration. For example, Doll *et al*[17], in a much cited paper, based on data for British doctors who started smoking at ages 16-25 and smoked 40 or less per day, modelled the annual lung cancer incidence at age 40-79 by the expression

$0.273 \times 10^{12} \times (\text{cigarettes/d} + 6)^2 \times (\text{age} - 22.5)^{4.5}$.

They noted "significant ($P < 0.01$) upward curvature of the dose-response relationship in the range 0-40 cigarettes/d, which is what might be expected if more than one of the 'stages' (in the multistage genesis of bronchial carcinoma) was strongly affected by smoking." They also noted a drop off in response above 40 cigarettes/d, though based on few cases, and discussed various explainations for it. Our analyses show little evidence of upward curvature with amount smoked. However, this does not rule out smoking affecting more than one stage of a multistage process; indeed there is strong evidence this is true[18].

Taking (age - 22.5 years) as an approximate indicator of duration, the model of Doll *et al*[17] suggests risk rises steeply with increasing duration, according to a fourth or fifth power relationship. At first sight, this appears to conflict with our findings, where the power relationship we fitted was only somewhat above linear (Figure 1C). However, whereas Doll and Peto's analysis compares risk by age for people of a similar age of start, our modelling compares risk by age of start for people of a given age. Here, the relationship of risk to duration will be much less steep. This can be illustrated by applying formulae for a form of the multistage model where risk affects the first and penultimate stages, the effect on the penultimate stage being twice as strong as for the first stage, a form known to fit smoking and lung cancer relationships quite well[18,19]. The RR for a 70-year-old starting at age 15 is estimated as 1.66 times higher than for a 70-year-old starting at age 30. This ratio somewhat exceeds the ratio of durations (55/40 = 1.38), but much less than predicted by a fourth or fifth power relationship ($1.38^{4.5} = 4.26$).

Summing up

Based on 71 studies described in 87 publications[20-106] we demonstrated that for all three measures of dose studied (amount smoked, duration and age of start), the shape of their relationship with lung cancer can be described quite accurately using simple models. Though, for all dose measures, there is evidence of misfit for some data blocks, these seem mainly due to unusual response patterns difficult to fit with plausible models, or to different blocks showing differing shapes of the dose-relationship. The main limitations of the models relate to the data they were fitted to. Misclassification of smoking status and of dose may produce bias, as may failure to update smoking habits during follow-up in prospective studies, and failure to adjust for other indices of dose. Nevertheless, the models presented characterize the observed relationships of lung cancer to amount smoked, duration, and age of start more fully than previously attempted.

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COMMENTS

Background

No previous meta-analysis has used parametric models in order to quantify more precisely the relationship between smoking and lung cancer. Using a database of all epidemiological studies of 100 or more lung cancer cases published before 2000, models are fitted relating lung cancer risk to amount smoked, duration of smoking and age of starting to smoke.

Research frontiers

Based on all the studies providing relevant data, the models fitted show that the risk, relative to never smokers, rises from 3.86 to 22.31 between 10 and 50 cigarettes/d, from 2.21 to 13.54 between 10 and 50 years smoked, and from 3.66 to 8.94 between age of starting 30 and 12.5 years. There is little heterogeneity between studies for duration of smoking or age started, but there is clear heterogeneity for amount smoked, with RRs for 50 cigarettes/d being 7.23 for studies in Asia, as compared to 26.36 for North American and 22.16 for European studies.

Innovations and breakthroughs

The new feature of this paper is the comprehensive assessment of the shape of the dose-responses studied, with a number of alternative functional forms studied, and the best-fitting one selected. The fitted models, which describe the relationships well, are each quite simple in form, allowing ready meta-analysis of individual study estimates.

Applications

The fitted models allow more precise quantification of the hazards of smoking than previously reported, and will assist smoking and health researchers.

Terminology

Linear model: The logarithm of the RR is linearly related to dose. In the power model it is related to dose raised to a power. In the log-with-baseline model, it is related to the logarithm of dose with an offset for background risk. Pseudo-numbers are estimates of numbers of cases and controls, by dose level, derived from published RRs, which allow fitting of the models.

Peer review

The authors meta-analyzed smoking/lung cancer relationships using parametric modelling according to the IESLC database. They found that the models describe the dose-relationship well and concluded that they can be used to more precisely estimate the lung cancer risk from smoking. The limitation has been fully discussed in the discussion part. This study provides some interesting results for further research into smoking and lung cancer.

REFERENCES

1 **Lee PN**, Forey BA, Coombs KJ. Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer. *BMC Cancer* 2012; **12**: 385 [PMID: 22943444 DOI: 10.1186/1471-2407-12-385]

2 **Fry JS**, Lee PN, Forey BA, Coombs KJ. How rapidly does the excess risk of lung cancer decline following quitting smoking? A quantitative review using the negative exponential model. *Regul Toxicol Pharmacol* 2013; **67**: 13-26 [PMID: 23764305 DOI: 10.1016/j.yrtph.2013.06.001]

3 **Fry JS**, Lee PN. Revisiting the association between environmental tobacco smoke exposure and lung cancer risk. I. The dose-response relationship with amount and duration of smoking by the husband. *Indoor Built Environ* 2000; **9**: 303-316 [DOI: 10.1177/1420326X0000900602]

4 **Hamling J**, Lee P, Weitkunat R, Ambühl M. Facilitating meta-analyses by deriving relative effect and precision estimates for alternative comparisons from a set of estimates presented by exposure level or disease category. *Stat Med* 2008; **27**: 954-970 [PMID: 17676579 DOI: 10.1002/sim.3013]

5 Thun MJ, Day-Lally C, Myers DG, Calle EE, Flanders WD, Zhu BP, Namboodiri MM, Heath CW Jr. Trends in tobacco smoking and mortality from cigarette use in cancer prevention studies I (1959 through 1965) and II (1982 through 1988). In: Shopland DR, Burns DM, Garfinkel L, Samet JM, editors. Changes in cigarette-related disease risks and their implications for prevention and control. Rockville, Maryland: US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 1997: 305-382. Available from: URL: http://cancercontrol.cancer.gov/tcrb/monographs/8/m8_4.pdf

6 **Forey B**, Hamling J, Lee P, Wald N. International Smoking Statistics: A collection of historical data from 30 economically developed countries. 2nd ed. London and Oxford: Wolfson Institute of Preventive Medicine and Oxford University Press, 2002 [DOI: 10.1093/acprof:oso/9780198508564.001.0001]

7 **Forey B**, Hamling J, Lee P. International Smoking Statistics. A collection of worldwide historical data. 2nd ed. Sutton: P N Lee Statistics and Computing Ltd., 2006-2012. Available from: URL: http://www.pnlee.co.uk/iss.htm

8 **US Department of Health and Human Services**. National health and nutrition examination survey (NHANES). National Center for Health Statistics. Available from: URL: http://www.cdc.gov/nchs/nhanes.htm

9 **Greenland S**, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* 1992; **135**: 1301-1309 [PMID: 1626547]

10 **Berlin JA**, Longnecker MP, Greenland S. Meta-analysis of epidemiologic dose-response data. *Epidemiology* 1993; **4**: 218-228 [PMID: 8512986 DOI: 10.1097/00001648-199305000-00005]

11 **http://www.pnlee.co.uk/downloads/ieslc3/lc_dose_response_paper_additionalfiles.htm**

12 **Lee PN**. Misclassification of smoking habits and passive smoking. A review of the evidence. Heidelberg: Springer-Verlag, 1988 [DOI: 10.1007/978-3-642-73822-7]

13 **Lee PN**, Forey BA. Misclassification of smoking habits as determined by cotinine or by repeated self-report - a summary of evidence from 42 studies. *J Smoking-Related Dis* 1995; **6**: 109-129

14 **Lee PN**, Forey B, Fry JS. Revisiting the association between environmental tobacco smoke exposure and lung cancer risk. III. Adjustment for the biasing effect of misclassification of smoking habits. *Indoor Built Environ* 2001; **10**: 384-398 [DOI: 10.1177/1420326X0101000605]

15 **Baker RR**, Dixon M, Mariner DC, Shepperd CJ, Scherer G, Ogden MW, Robinson JH, Sinclair NM, Sherwood N, Akiyama Y, Sakamoto K, Röper W, Tricker AR, Marchand V, Varignon B, Lionetti G. Terms used for exposure to smoke. *Beitr Tabakforsch Int* 2004; **21**: 250

16 **Orsini N**, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. *Am J Epidemiol* 2012; **175**: 66-73 [PMID: 22135359]

17 **Doll R**, Peto R. Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong non-smokers. *J Epidemiol Community Health* 1978; **32**: 303-313 [PMID: 744822 DOI: 10.1136/jech.32.4.303]

18 **Lee PN**. Studying the relationship of smoking to lung cancer using the multistage model of carcinogenesis. A review. Sutton, Surrey: P N Lee Statistics and Computing Ltd., 1995. Available from: URL: http://www.pnlee.co.uk/Reports.htm

19 **Day NE**, Brown CC. Multistage models and primary prevention of cancer. *J Natl Cancer Inst* 1980; **64**: 977-989 [PMID: 6929006]

20 **Akiba S**. Analysis of cancer risk related to longitudinal information on smoking habits. *Environ Health Perspect* 1994; **102** Suppl 8: 15-19 [PMID: 7851325 DOI: 10.1289/ehp.94102s815]

21 **Amandus H**, Costello J. Silicosis and lung cancer in U.S. metal miners. *Arch Environ Health* 1991; **46**: 82-89 [PMID: 2006898 DOI: 10.1080/00039896.1991.9937433]

22 **Archer VE**, Gillam JD, Wagoner JK. Respiratory disease mortality among uranium miners. *Ann NY Acad Sci* 1976; **271**: 280-293 [DOI: 10.1111/j.1749-6632.1976.tb23123.x]

23 **Rylander R**, Axelsson G, Andersson L, Liljequist T, Bergman B. Lung cancer, smoking and diet among Swedish men. *Lung Cancer* 1996; **14** Suppl 1: S75-S83 [PMID: 8785669 DOI: 10.1016/S0169-5002(96)90212-3]

24 **Modigh C**, Axelsson G, Alavanja M, Andersson L, Rylander R. Pet birds and risk of lung cancer in Sweden: a case-control study. *BMJ* 1996; **313**: 1236-1238 [PMID: 8939112 DOI: 10.1136/bmj.313.7067.1236]

25 **Ben-Shlomo Y**, Smith GD, Shipley MJ, Marmot MG. What determines mortality risk in male former cigarette smokers? *Am J Public Health* 1994; **84**: 1235-1242 [PMID: 8059878 DOI: 10.2105/AJPH.84.8.1235]

26 **Marmot MG**, Shipley MJ, Rose G. Inequalities in death--specific explanations of a general pattern? *Lancet* 1984; **1**: 1003-1006 [PMID: 6143919]

27 **Department of National Health and Welfare Canada**. A Canadian study of smoking and health. Canada: Department of National Health and Welfare, 1966

28 **Boucot KR**, Weiss W, Seidman H, Carnahan WJ, Cooper DA. The Philadelphia pulmonary neoplasm research project: basic risk factors of lung cancer in older men. *Am J Epidemiol* 1972; **95**: 4-16 [PMID: 5007365]

29 **Brett GZ**, Benjamin B. Smoking habits of men employed in industry, and mortality. *Br Med J* 1968; **3**: 82-85 [PMID: 5662965 DOI: 10.1136/bmj.3.5610.82]

30 **Bross ID**, Gibson R. Risks of lung cancer in smokers who switch to filter cigarettes. *Am J Public Health Nations Health* 1968; **58**: 1396-1403 [PMID: 5691372 DOI: 10.2105/AJPH.58.8.1396]

31 **Buffler PA**, Pickle LW, Mason TJ, Contant C. The causes of lung cancer in Texas. In: Mizell M, Correa P, editors. Lung cancer: causes and prevention, Proceedings of the International Lung Cancer Update Conference, New Orleans, Louisiana, March 3-5, 1983. Deerfield Beach, FL: Verlag Chemie International Inc., 1984: 83-99

32 **Ives JC**. Environmental exposures and lung cancer risk among women in Harris County, Texas, 1977-1980 [Thesis]. Houston, TX: University of Texas, Health Science Centre, 1984

33 **Cederlöf R**, Friberg L, Hrubec Z, Lorich U. The relationship of smoking and some social covariables to mortality and cancer morbidity. A ten year follow-up in a probability sample of 55,000 Swedish subjects age 18-69, Part 1/2. Stockholm:

Karolinska Institute, Dept of Environmental Hygiene, 1975
34 **Nordlund LA**, Carstensen JM, Pershagen G. Cancer incidence in female smokers: a 26-year follow-up. *Int J Cancer* 1997; **73**: 625-628 [PMID: 9398036]
35 **Chang AK**, Barrett-Connor E, Edelstein S. Low plasma cholesterol predicts an increased risk of lung cancer in elderly women. *Prev Med* 1995; **24**: 557-562 [PMID: 8610078 DOI: 10.1006/pmed.1995.1089]
36 **Chow WH**, Schuman LM, McLaughlin JK, Bjelke E, Gridley G, Wacholder S, Chien HT, Blot WJ. A cohort study of tobacco use, diet, occupation, and lung cancer mortality. *Cancer Causes Control* 1992; **3**: 247-254 [PMID: 1610971 DOI: 10.1007/BF00124258]
37 **Comstock GW**, Alberg AJ, Huang HY, Wu K, Burke AE, Hoffman SC, Norkus EP, Gross M, Cutler RG, Morris JS, Spate VL, Helzlsouer KJ. The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, alpha-tocopherol, selenium, and total peroxyl radical absorbing capacity. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 907-916 [PMID: 9367064]
38 **Correa P**, Pickle LW, Fontham E, Dalager N, Lin Y, Haenszel W, Johnson WD. The causes of lung cancer in Louisiana. In: Mizell M, Correa P, editors. Lung cancer: causes and prevention. New York: Verlag Chemie International Inc., 1984: 73-82
39 **Fontham ET**, Pickle LW, Haenszel W, Correa P, Lin YP, Falk RT. Dietary vitamins A and C and lung cancer risk in Louisiana. *Cancer* 1988; **62**: 2267-2273 [PMID: 3179940]
40 **Burns DM**, Shanks TG, Choi W, Thun MJ, Heath CW Jr, Garfinkel L. The American Cancer Society cancer prevention study I: 12-year follow-up of 1 million men and women. In: Changes in cigarette-related disease risks and their implications for prevention and control. Rockville, MD: US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 1997: 113-304. Available from: URL: http://cancercontrol.cancer.gov/tcrb/monographs/8/m8_3.pdf
41 **Hammond EC**. Smoking habits and air pollution in relation to lung cancer. In: Lee HK, editor. Environmental factors in respiratory diseases. New York: Academic Press Inc., 1972: 177-198
42 **Thun MJ**, Myers DG, Day-Lally C, Namboodiri MM, Calle EE, Flanders WD, Adams SL, Heath CW Jr. Age and the exposure-response relationships between cigarette smoking and premature death in Cancer Prevention Study II. In: Changes in cigarette-related disease risks and their implications for prevention and control. Rockville, MD: US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 1997: 383-475. Available from: URL: http://cancercontrol.cancer.gov/tcrb/monographs/8/m8_5.pdf
43 **Darby S**, Whitley E, Silcocks P, Thakrar B, Green M, Lomas P, Miles J, Reeves G, Fearn T, Doll R. Risk of lung cancer associated with residential radon exposure in south-west England: a case-control study. *Br J Cancer* 1998; **78**: 394-408 [PMID: 9703290 DOI: 10.1038/bjc.1998.506]
44 **Wicken AJ**. Environmental and personal factors in lung cancer and bronchitis mortality in Northern Ireland, 1960-1962. London: Tobacco Research Council, 1966
45 **Dean G**, Lee PN, Todd GF, Wicken AJ. Report on a second retrospective mortality study in North-East England - Part I. Factors related to mortality from lung cancer, bronchitis, heart disease and stroke in Cleveland County, with particular emphasis on the relative risks associated with smoking filter and plain cigarettes. London: Tobacco Research Council, 1977
46 **de Klerk NH**, Musk AW. Silica, compensated silicosis, and lung cancer in Western Australian goldminers. *Occup Environ Med* 1998; **55**: 243-248 [PMID: 9624278 DOI: 10.1136/oem.55.4.243]
47 **Doll R**, Peto R, Wheatley K, Gray R, Sutherland I. Mortality in relation to smoking: 40 years' observations on male British doctors. *BMJ* 1994; **309**: 901-911 [PMID: 7755693 DOI: 10.1136/bmj.309.6959.901]
48 **Doll R**, Peto R. Mortality in relation to smoking: 20 years' observations on male British doctors. *Br Med J* 1976; **2**: 1525-1536 [PMID: 1009386 DOI: 10.1136/bmj.2.6051.1525]
49 **Doll R**, Gray R, Hafner B, Peto R. Mortality in relation to smoking: 22 years' observations on female British doctors. *Br Med J* 1980; **280**: 967-971 [PMID: 7417764 DOI: 10.1136/bmj.280.6219.967]
50 **Dorant E**, van den Brandt PA, Goldbohm RA. A prospective cohort study on Allium vegetable consumption, garlic supplement use, and the risk of lung carcinoma in The Netherlands. *Cancer Res* 1994; **54**: 6148-6153 [PMID: 7954460]
51 **Dorgan JF**, Ziegler RG, Schoenberg JB, Hartge P, McAdams MJ, Falk RT, Wilcox HB, Shaw GL. Race and sex differences in associations of vegetables, fruits, and carotenoids with lung cancer risk in New Jersey (United States). *Cancer Causes Control* 1993; **4**: 273-281 [PMID: 8318643]
52 **McLaughlin JK**, Hrubec Z, Blot WJ, Fraumeni JF. Smoking and cancer mortality among U.S. veterans: a 26-year follow-up. *Int J Cancer* 1995; **60**: 190-193 [PMID: 7829214 DOI: 10.1002/ijc.2910600210]
53 **Enstrom JE**. Smoking cessation and mortality trends among two United States populations. *J Clin Epidemiol* 1999; **52**: 813-825 [PMID: 10529023]
54 **Kahn HA**. The Dorn study of smoking and mortality among U.S. veterans: report on eight and one-half years of observation. In: Haenszel W, editor. Epidemiological approaches to the study of cancer and other chronic diseases. Bethesda, MD: U.S. Department of Health, Education, and Welfare. Public Health Service National Cancer Institute, 1966: 1-125
55 **Engeland A**, Haldorsen T, Andersen A, Tretli S. The impact of smoking habits on lung cancer risk: 28 years' observation of 26,000 Norwegian men and women. *Cancer Causes Control* 1996; **7**: 366-376 [PMID: 8734831 DOI: 10.1007/BF00052943]
56 **Enstrom JE**, Heath CW. Smoking cessation and mortality trends among 118,000 Californians, 1960-1997. *Epidemiology* 1999; **10**: 500-512 [PMID: 10468422 DOI: 10.1097/00001648-199909000-00007]
57 **Gao CM**, Tajima K, Kuroishi T, Hirose K, Inoue M. Protective effects of raw vegetables and fruit against lung cancer among smokers and ex-smokers: a case-control study in the Tokai area of Japan. *Jpn J Cancer Res* 1993; **84**: 594-600 [PMID: 8340248 DOI: 10.1111/j.1349-7006.1993.tb02018.x]
58 **Gillis CR**, Hole DJ, Boyle P. Cigarette smoking and male lung cancer in an area of very high incidence. I. Report of a case-control study in the West of Scotland. *J Epidemiol Community Health* 1988; **42**: 38-43 [PMID: 3418284 DOI: 10.1136/jech.42.1.38]
59 **Haenszel W**, Shimkin MB, Mantel N. A retrospective study of lung cancer in women. *J Natl Cancer Inst* 1958; **21**: 825-842 [PMID: 13599015]
60 **Hammond EC**, Selikoff IJ, Seidman H. Asbestos exposure, cigarette smoking and death rates. *Ann N Y Acad Sci* 1979; **330**: 473-490 [PMID: 294198 DOI: 10.1111/j.1749-6632.1979.tb18749.x]
61 **Hammond EC**, Horn D. Smoking and death rates; report on forty-four monghs of follow-up of 187,783 men. II. Death rates by cause. *J Am Med Assoc* 1958; **166**: 1294-1308 [PMID: 13513355]
62 **Hirayama T**. Life-style and mortality: A large scale census based cohort study in Japan. In: Wahrendorf J, editor. Contributions to epidemiology and biostatistics. Basle: Karger, 1990: 6
63 **Hitosugi M**. Epidemiological study of lung cancer with special reference to the effect of air pollution and smoking habit. *Bull Inst Public Health* 1968; **17**: 237-256
64 **Gillis CR**, Hole DJ, Hawthorne VM. Cigarette smoking and

male lung cancer in an area of very high incidence. II. Report of a general population cohort study in the West of Scotland. *J Epidemiol Community Health* 1988; **42**: 44-48 [PMID: 3418285 DOI: 10.1136/jech.42.1.44]
65 **Humble CG**, Samet JM, Pathak DR, Skipper BJ. Cigarette smoking and lung cancer in 'Hispanic' whites and other whites in New Mexico. *Am J Public Health* 1985; **75**: 145-148 [PMID: 3966619 DOI: 10.2105/AJPH.75.2.145]
66 **Pathak DR**, Samet JM, Humble CG, Skipper BJ. Determinants of lung cancer risk in cigarette smokers in New Mexico. *J Natl Cancer Inst* 1986; **76**: 597-604 [PMID: 3457198]
67 **Friedman GD**, Tekawa I, Sadler M, Sidney S. Smoking and mortality: the Kaiser Permanente experience. In: Changes in cigarette-related disease risks and their implications for prevention and control. Rockville, MD: US Department of Health and Human Services, National Institutes of Health, National Cancer Institute, 1997: 477-499. Available from: URL: http://cancercontrol.cancer.gov/tcrb/monographs/8/m8_6.pdf
68 **Selby JV**, Friedman GD. Epidemiologic evidence of an association between body iron stores and risk of cancer. *Int J Cancer* 1988; **41**: 677-682 [PMID: 3366489 DOI: 10.1002/ijc.2910410507]
69 **Kanellakis A**, Trichopoulos D, Michalakopoulos N, Margoudakis S, Kanellaki K, Xirouchaki E, Kalapothaki V. The relationship between smoking of Greek cigarettes and the development of lung cancer. *Mater Med Greca* 1976; **4**: 351-355
70 **Trichopoulos D**, Kalandidi A, Tzonou A. Incidence and distribution of lung cancer in Greece. *Excerpta Med Int Congr Ser* 1982; **558**: 10-17
71 **Katsouyanni K**, Trichopoulos D, Kalandidi A, Tomos P, Riboli E. A case-control study of air pollution and tobacco smoking in lung cancer among women in Athens. *Prev Med* 1991; **20**: 271-278 [PMID: 2057473 DOI: 10.1016/0091-7435(91)90026-Z]
72 **Kaufman DW**, Palmer JR, Rosenberg L, Stolley P, Warshauer E, Shapiro S. Tar content of cigarettes in relation to lung cancer. *Am J Epidemiol* 1989; **129**: 703-711 [PMID: 2923118]
73 **Kinlen LJ**, Willows AN, Goldblatt P, Yudkin J. Tea consumption and cancer. *Br J Cancer* 1988; **58**: 397-401 [PMID: 3179194 DOI: 10.1038/bjc.1988.227]
74 **Knekt P**. Vitamin E and smoking and the risk of lung cancer. *Ann N Y Acad Sci* 1993; **686**: 280-27; discussion 280-27; [PMID: 8512253 DOI: 10.1111/j.1749-6632.1993.tb39187.x]
75 **Knekt P**, Seppänen R, Järvinen R, Virtamo J, Hyvönen L, Pukkala E, Teppo L. Dietary cholesterol, fatty acids, and the risk of lung cancer among men. *Nutr Cancer* 1991; **16**: 267-275 [PMID: 1775388 DOI: 10.1080/01635589109514165]
76 **Koo LC**, Ho JHC, Saw D. Is passive smoking an added risk factor for lung cancer in Chinese women? *J Exp Clin Cancer Res* 1984; **3**: 277-283
77 **Koo LC**, Ho JHC, Saw D. Active and passive smoking among female lung cancer patients and controls in Hong Kong. *J Exp Clin Cancer Res* 1983; **2**: 367-375
78 **Liaw KM**, Chen CJ. Mortality attributable to cigarette smoking in Taiwan: a 12-year follow-up study. *Tob Control* 1998; **7**: 141-148 [PMID: 9789932 DOI: 10.1136/tc.7.2.141]
79 **McDonald JC**, Liddell FD, Dufresne A, McDonald AD. The 1891-1920 birth cohort of Quebec chrysotile miners and millers: mortality 1976-88. *Br J Ind Med* 1993; **50**: 1073-1081 [PMID: 8280638 DOI: 10.1136/oem.50.12.1073]
80 **MacLennan R**, Da Costa J, Day NE, Law CH, Ng YK, Shanmugaratnam K. Risk factors for lung cancer in Singapore Chinese, a population with high female incidence rates. *Int J Cancer* 1977; **20**: 854-860 [PMID: 591126 DOI: 10.1002/ijc.2910200606]
81 **Matos E**, Vilensky M, Boffetta P, Kogevinas M. Lung cancer and smoking: a case-control study in Buenos Aires, Argentina. *Lung Cancer* 1998; **21**: 155-163 [PMID: 9857993 DOI: 10.1016/S0169-5002(98)00055-5]
82 **Lee PN**. Mortality from smoking-associated diseases in Great Britain. A statistical analysis of British data from the U.S.A.-U.K.-Norway migrant study. Sutton, Surrey: P N Lee Statistics and Computing Ltd., 1979. Available from: URL: http://www.pnlee.co.uk/Reports.htm
83 **Kuller LH**, Ockene JK, Meilahn E, Wentworth DN, Svendsen KH, Neaton JD. Cigarette smoking and mortality. MRFIT Research Group. *Prev Med* 1991; **20**: 638-654 [PMID: 1758843 DOI: 10.1016/0091-7435(91)90060-H]
84 **Kuller LH**, Ockene J, Meilahn E, Svendsen KH. Relation of forced expiratory volume in one second (FEV1) to lung cancer mortality in the Multiple Risk Factor Intervention Trial (MRFIT). *Am J Epidemiol* 1990; **132**: 265-274 [PMID: 2372006]
85 **Nam CB**, Hummer RA, Rogers RG. Underlying and multiple causes of death related to smoking. *Popul Res Policy Rev* 1994; **13**: 305-325 [DOI: 10.1007/BF01074340]
86 **Parkin DM**, Vizcaino AP, Skinner ME, Ndhlovu A. Cancer patterns and risk factors in the African population of southwestern Zimbabwe, 1963-1977. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 537-547 [PMID: 7827583]
87 **Pershagen G**, Akerblom G, Axelson O, Clavensjö B, Damber L, Desai G, Enflo A, Lagarde F, Mellander H, Svartengren M. Residential radon exposure and lung cancer in Sweden. *N Engl J Med* 1994; **330**: 159-164 [PMID: 8264737 DOI: 10.1056/NEJM199401203300302]
88 **Peto R**, Speizer FE, Cochrane AL, Moore F, Fletcher CM, Tinker CM, Higgins IT, Gray RG, Richards SM, Gilliland J, Norman-Smith B. The relevance in adults of air-flow obstruction, but not of mucus hypersecretion, to mortality from chronic lung disease. Results from 20 years of prospective observation. *Am Rev Respir Dis* 1983; **128**: 491-500 [PMID: 6614643]
89 **Pezzotto SM**, Poletto L. Occupation and histopathology of lung cancer: A case-control study in Rosario, Argentina. *Am J Ind Med* 1999; **36**: 437-443 [PMID: 10470008]
90 **Pezzotto SM**, Mahuad R, Bay ML, Morini JC, Poletto L. Variation in smoking-related lung cancer risk factors by cell type among men in Argentina: a case-control study. *Cancer Causes Control* 1993; **4**: 231-237 [PMID: 8391337]
91 **Prescott E**, Osler M, Hein HO, Borch-Johnsen K, Lange P, Schnohr P, Vestbo J. Gender and smoking-related risk of lung cancer. The Copenhagen Center for Prospective Population Studies. *Epidemiology* 1998; **9**: 79-83 [PMID: 9430273 DOI: 10.1097/00001648-199801000-00016]
92 **Segi M**, Kurihara M, Ishikawa S, Haenszel W. Epidemiological survey on lung cancer and smoking. *Lung Cancer* 1979; **19**: 157-165 [DOI: 10.2482/haigan.19.157]
93 **Shaw GL**, Falk RT, Deslauriers J, Frame JN, Nesbitt JC, Pass HI, Issaq HJ, Hoover RN, Tucker MA. Debrisoquine metabolism and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 1995; **4**: 41-48 [PMID: 7894323]
94 **Sobue T**, Suzuki T, Fujimoto I, Matsuda M, Doi O, Mori T, Furuse K, Fukuoka M, Yasumitsu T, Kuwahara O. Case-control study for lung cancer and cigarette smoking in Osaka, Japan: comparison with the results from Western Europe. *Jpn J Cancer Res* 1994; **85**: 464-473 [PMID: 8014103 DOI: 10.1111/j.1349-7006.1994.tb02381.x]
95 **Speizer FE**, Colditz GA, Hunter DJ, Rosner B, Hennekens C. Prospective study of smoking, antioxidant intake, and lung cancer in middle-aged women (USA). *Cancer Causes Control* 1999; **10**: 475-482 [PMID: 10530619 DOI: 10.1023/A:1008931526525]
96 **Stockwell HG**, Lyman GH, Waltz J, Peters JT. Lung cancer in Florida. Risks associated with residence in the central Florida phosphate mining region. *Am J Epidemiol* 1988; **128**: 78-84 [PMID: 2837899]
97 **Svensson C**, Pershagen G, Klominek J. Smoking and passive smoking in relation to lung cancer in women. *Acta Oncol* 1989; **28**: 623-629 [PMID: 2590538 DOI: 10.3109/02841868909092282]

98 **Tenkanen L**, Hakulinen T, Teppo L. The joint effect of smoking and respiratory symptoms on risk of lung cancer. *Int J Epidemiol* 1987; **16**: 509-515 [PMID: 3440661 DOI: 10.1093/ije/16.4.509]
99 **Tenkanen L**, Hakulinen T, Hakama M, Saxén E. Sauna, dust and migration as risk factors in lung cancer among smoking and non-smoking males in Finland. *Int J Cancer* 1985; **35**: 637-642 [PMID: 3997283]
100 **Tsugane S**, Watanabe S, Sugimura H, Arimoto H, Shimosato Y, Suemasu K. Smoking, occupation and family history in lung cancer patients under fifty years of age. *Jpn J Clin Oncol* 1987; **17**: 309-317 [PMID: 2826845]
101 **Tulinius H**, Sigfússon N, Sigvaldason H, Bjarnadóttir K, Tryggvadóttir L. Risk factors for malignant diseases: a cohort study on a population of 22,946 Icelanders. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 863-873 [PMID: 9367058]
102 **Tverdal A**, Thelle D, Stensvold I, Leren P, Bjartveit K. Mortality in relation to smoking history: 13 years' follow-up of 68,000 Norwegian men and women 35-49 years. *J Clin Epidemiol* 1993; **46**: 475-487 [PMID: 8501474 DOI: 10.1016/0895-4356(93)90025-V]
103 **Wakai K**, Ohno Y, Genka K, Ohmine K, Kawamura T, Tamakoshi A, Aoki R, Kojima M, Lin Y, Aoki K, Fukuma S. Smoking habits, local brand cigarettes and lung cancer risk in Okinawa, Japan. *J Epidemiol* 1997; **7**: 99-105 [PMID: 9255031 DOI: 10.2188/jea.7.99]
104 **Wu AH**, Henderson BE, Pike MC, Yu MC. Smoking and other risk factors for lung cancer in women. *J Natl Cancer Inst* 1985; **74**: 747-751 [PMID: 3857370]
105 **Harris RE**, Zang EA, Anderson JI, Wynder EL. Race and sex differences in lung cancer risk associated with cigarette smoking. *Int J Epidemiol* 1993; **22**: 592-599 [PMID: 8225730 DOI: 10.1093/ije/22.4.592]
106 **Yamaguchi N**, Kido M, Hoshuyama T, Manabe H, Kikuchi Y, Nishio T, Ohshima LH, Watanabe S. A case-control study on occupational lung cancer risks in an industrialized city of Japan. *Jpn J Cancer Res* 1992; **83**: 134-140 [PMID: 1555994 DOI: 10.1111/j.1349-7006.1992.tb00077.x]

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META-ANALYSIS

Ophthalmic adverse drug reactions: A nationwide detection using hospital databases

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Abstract

AIM: To detect ophthalmic adverse drug reactions (ADRs), that occurred in Portugal from 2000 to 2009, through the utilization of administrative hospital databases. We also intended to compare the results of this methodology with spontaneous reporting.

METHODS: We conducted a retrospective nationwide study using hospital administrative databases, which included all inpatients and outpatients in all public hospitals in Portugal, from 2000 to 2009. We used International Classification of Diseases - 9th Revision - Clinical Modification (ICD-9-CM) coding data that allowed the detection of ADRs. We used WHO's definition for ADR. We searched all of ICD-9-CM terms in Ophthalmology for codes that included "drug-induced", "iatrogenic", "toxic" and all other that could signal an ADR, such as "362.55 - toxic maculopathy" or "365.03 - steroid responders", and also "E" codes (codes from E930 to E949.9, that exclude intoxications and errors).

RESULTS: From 11944725 hospitalizations or ambulatory episodes within that period of time, we identified 1524 probable ophthalmic ADRs (corresponding to a frequency of 1.28 per 10000 episodes) and an additional 100 possible ophthalmic ADRs. We used only 4 person-hours in the application of this methodology. A total of 113 spontaneous reports arose from ophthalmic ADRs from 2000 to 2009 in Portugal (frequency of 0.095 per 10000 episodes).To our knowledge, this was the first estimate of the frequency of ophthalmic ADRs through the use of databases, and the first nationwide estimate of ophthalmic ADRs, in Portugal. We identified 1524 probable ADRs and 100 possible ADRs.

CONCLUSION: This database methodology adapted for Ophthalmology may represent a new approach for the detection of ophthalmic ADRs, since these codes exist in the ICD-9-CM classification. Its performance was clearly superior to spontaneous reporting.

Key words: Adverse drug reactions; Ophthalmology; Ocular; Databases; Pharmacovigilance

Core tip: We used International Classification of Diseases - 9th Revision - Clinical Modification coding data for the detection of adverse drug reactions (ADRs). From 11944725 episodes, we identified 1524 probable ophthalmic ADRs. 113 spontaneous reports arose from that population. This was the first nationwide study of ophthalmic ADRs and may represent a new Pharmacovigilance approach, with a higher detection than spontaneous reporting.

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INTRODUCTION

Adverse drug reactions (ADRs) are responsible for significant morbidity, mortality and costs in Health Care systems[1]. They may occur in 16.9% of patients during hospitalization (95%CI: 13.5-20.2)[2] and provoke 5.3% of hospital admissions (interquartile range 2.7%-9.0%)[3]. ADRs are a frequent cause of death in developed countries[4]. However, in Ophthalmology the evidence is scarce and lacks systematization[5]. A review about challenges in ADRs in Ophthalmology[5] concluded that there are several areas that can be improved, namely by applying always the definition of ADR of the World Health Organization (WHO)[6], by performing a causality assessment in each ADR (which determines the probability of representing a true ADR; the most utilized causality assessments of ADRs are from WHO[7] and from Naranjo *et al*[8]).

The development and validation of new methodologies for an improved detection of ADRs would be another area of improvement[5,9]. There are Pharmacovigilance methodologies[9] used for the detection of ADRs and that can be adapted for detecting ADRs in Ophthalmology, but they may have methodological issues: Spontaneous reporting is the most used (it needs low resources) and is the only Pharmacovigilance method continuously used in the majority of countries, being the main support of WHO International Drug Program. However, it has several limitations, namely, the smallest detection rate of several Pharmacovigilance methods[10], under-reporting[11], heterogeneous report quality[12] and increased risk of bias[12]. Intensive and prospective monitoring are methodologies with good detection rates but too resource-consuming for continuous application[13].

Administrative hospital databases have large clinical information and thus may represent an interesting Pharmacovigilance approach with readily available and cheap information[10]. Some authors have utilized databases[10,14] for the detection of ADRs, taking advantage of the large quantity of clinical information readily available, containing coding data that can be used as an alert for the detection of an ADR, with low relatively low resources required.

Our purpose was to identify and characterize ophthalmic ADRs in a Nationwide study in Portugal, using hospital databases with clinical information.

MATERIALS AND METHODS

Study design

A retrospective study was performed for ADR identification using hospital administrative databases with information from all public hospitals in Portugal, from 2000 to 2009, obtained from our National Health Department (data from the second semester of 2009 was not available). These databases contain anonymized data for patient identification, episode and process number, and also information on age, sex, admission date, discharge date, ward(s), hospital attended (tertiary, university), area of Healthcare, district, outcome (death, discharge, transfer), payment data and International Classification of Diseases - 9th Revision - Clinical Modification (ICD-9-CM)[15] codes for: diagnoses (principal diagnosis, other diagnosis up to 19), procedures (up to 20) and external causes (up to 20). Patient population included all patients hospitalized or admitted for ambulatory care, in all public hospitals in Portugal, from 2000 to 2009 (inpatients and outpatients). All investigations were performed according to the guidelines of the Declaration of Helsinki and Institutional Review Board approval from was obtained.

Definition of ADR

There is some misuse of terms in this matter; therefore we present definitions.

An ADR[6] is: "any noxious, unintended and undesired effect of a drug, which occurs at doses used in humans for prophylaxis, diagnosis, or therapy". Therefore, to increase specificity, we wanted to assess only ADRs. Adverse drug event is not a synonym of ADR. There are other definitions of ADR, namely from Karch *et al*[16] and from Edwards *et al*[17], but we used the definition of WHO. An adverse event[18] is: "an injury related to medical management (all aspects of care, including diagnosis and treatment, failure to diagnose or treat, and the systems and equipment used to deliver care), in contrast to complications of disease". An adverse drug event[19] is: "An injury related to the use of a drug, although the causality of this relationship may not be proven". These include medication errors (namely the prescription of a wrong dose) and ADRs. We aimed to assess strictly ADRs.

Detection of ADRs

Hospital administrative databases include information of diagnosis. Codes searched for ADR identification were adapted to the specificities of Ophthalmology and resulted from a thorough search of: all terms of ICD-9-CM in Ophthalmology that included "drug-induced", "iatrogenic", "toxic" and all codes that could signal an ADR, such as "362.55 - toxic maculopathy" or "365.03 - steroid responsers", as detailed in the Results Section.

We also performed a search of general ADRs through the use of 'E' codes (ICD-9-CM codes from E930 to E949.9, designed to represent ADRs and already excluding wrong doses, errors and intoxications) to assess if these general ADRs could detect ophthalmic ADRs.

In this study, we performed a query of Ophthalmology in a nationwide study using administrative databases, including inpatients and ambulatory patients. Our main outcome was ADR detection. Secondary outcomes included: type of ADR, age, sex, admission diagnosis, other diagnoses, hospital length-of-stay and year of discharge. We performed WHO's causality assessments of ADRs,

with two independent reviewers. Differences were resolved by consensus. A third review was consulted to help resolved differences. We also registered how many person-hours were spent in the application of this methodology, to estimate cost (resources spent). The number of person-hours refers to the number of hours and number of people used in the application of this methodology; commonly used in the comparison of different Pharmacovigilance methodologies[19]. The number of spontaneous reporting of ADRs in hospitalized patients from 2000 to 2009 was obtained from Portuguese National Authority of Medicines (INFARMED), for comparison[20].

Statistical analysis

Statistical analyses were done using the χ^2 test for categorical variables (or exact Fisher's test whenever possible), Student's *t*-test for normally distributed continuous variables and Mann-Whitney or Kruskal-Wallis for variables without normal distribution, using SPSS v20. The a priori level of significance was $P < 0.05$.

RESULTS

Study population

There were 11944725 patients hospitalized or with ambulatory episodes in public hospitals of Portugal, from 2000 to the first semester of 2009. The baseline characteristics of the study population (n = 11944725) are shown in Table 1. The mean age of hospitalized patients was 48 ± 27 years and in 55.2% of episodes the patient was female. We spent only 4 person-hours in the application of this methodology.

From 2000, there was a slight increase in the number of hospitalizations in Portugal. Specific ophthalmic ADRs (n = 1524) were detected through the search of codes that could represent particular ophthalmic ADRs, as shown in Table 2. This corresponds to a frequency of 1.28 ophthalmic ADR per 10000 episodes. Additionally, 100 episodes that could possibly correspond to an ophthalmic ADR were also detected (Table 2). Therefore, a total of 1624 possible ophthalmic ADRs were detected. These possible ADRs included: conjunctival concretions, pigmentations and deposits (which can be caused by drugs such as topical adrenaline[21], but also by other factors, therefore may correspond to an ADR in some cases) and acquired color vision deficiencies (which may be caused by drugs such as sildenafil[22], but have other non related causes).

The search of general ADRs through the use of "E" codes allowed us to identify 116720 ADRs, but only 62 of them corresponded to the ophthalmic ADRs that were identified.

The total number of spontaneous notifications of ADRs in Portugal from 2000 to 2009 was 13562, from which 113 were spontaneous reports specific of ophthalmic ADRs. There were 553 additional spontaneous reports of systemic ADRs that included some ophthalmic manifestations.

Table 1 Socio-demographic characteristics of study population

Characteristic	Value
Number of episodes (inpatient, ambulatory)	11944725
Mean age (yr, mean ± SD)	48 ± 27
Female gender n (%)	6598266 (55.2)
District with higher number of hospitalizations	1st: Lisbon 21.2% 2nd: Oporto 17.2% 3rd: Setubal 7.66%
Mean hospital length-of-stay for inpatients (d, mean ± SD)	7.1 ± 3.21
Number of probable ophthalmic ADRs	1524

ADRs: Adverse drug reactions.

Table 2 Clinical codes searched and respective results in the portuguese database

ICD-9-CM code	Diagnosis	No. of episodes
Specific ophthalmic ADR codes		
362.55	Toxic maculopathy	1388
365.03	Steroid responders	4
365.31, 365.32	Corticosteroid-induced glaucoma	0
364.55	Miotic pupillary cyst (provoked by pilocarpine)	2
364.81	Floppy iris syndrome	2
366.45	Toxic cataract	83
367.89	Other drug-induced disorders of refraction and accommodation, Toxic disorders of refraction and accommodation	25
377.34	Toxic optic neuropathy, Toxic amblyopia	20
Possible signs of ophthalmic ADRs		
366.46	Cataract associated with radiation and other physical influences	10
372.54	Conjunctival concretions	67
372.55	Conjunctival pigmentations, including conjunctival argyrosis	
372.56	Conjunctival deposits	
368.55	Acquired color vision deficiencies	23
368.59	Other color vision deficiencies	
	Sub-Total specific	1524
	Total	1624

ICD-9-CM: Classification of Diseases - 9th Revision - Clinical Modification; ADRs: Adverse drug reactions.

DISCUSSION

To our knowledge, this is the first estimate of the frequency of ophthalmic ADRs through the use of administrative databases, and the first to apply a nationwide estimate of ophthalmic ADRs, in Portugal. We identified 1524 probable ADRs and 100 possible ADRs. This may represent a new approach for the detection of ophthalmic ADRs, since these codes exist in the ICD-9-CM classification.

The strengths of our study include: our comprehensive database, which contains data from all hospitalizations and ambulatory episodes in every public hospital in Portugal within almost a decade, the fact that this is a new methodology to aid ADR detection (until now only case reports and spontaneous reports were available for

ADR detection), and the fact that these codes are widely available and universal, making possible to easily build estimates of ophthalmic ADRs in other countries and other years. In fact, it would be very interesting to see if ophthalmic ADRs in Portugal have the same distribution, frequency and characteristics in comparison with other countries, therefore further studies are necessary.

Limitations of our work are inherent to the use of administrative databases, which may contain incomplete or wrong data and coding bias[23] (in which coders select a different code to increase reimbursement to their hospital). The small number of ADRs found may be considered a limitation, but on the other hand this is a methodology resource-sparing (only 4 person-hours spent in its application), having potential for widespread application in other countries. Also, this method identified 1524 probable ADRs, a much higher number than the number of ophthalmic ADRs found by spontaneous reporting: 113.

We suggest complementing spontaneous reporting with this database methodology to increase detection of ophthalmic ADRs. In fact, the complementary use of several methodologies is defended by several authors[24], in order to enhance ADR detection and increase patient safety. Finally, we believe that after this study, these codes should be applied prospectively in a future study in a nation-wide basis, enabling an expert to confirm each ADR and causing drug, to further complete and validate the data suggested here, and to integrate this method as a Pharmacovigilance methodology.

In conclusion, Ophthalmology represents simultaneously a challenge and an opportunity to identify ADRs. This is the first nationwide estimate of ophthalmic ADRs. Administrative databases are a useful methodology for the detection of ocular ADRs, but require adapted diagnoses codes. They may underestimate the real number of ADRs, but nevertheless they have the potential to complement spontaneous reporting as a methodology for ophthalmic ADR detection, with a higher detection rate.

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COMMENTS

Background

Adverse drug reactions (ADRs) are a frequent cause of death in developed countries. However, in Ophthalmology the evidence is scarce and lacks systematization.

Research frontiers

There are Pharmacovigilance methodologies used for the detection of ADRs and that can be adapted for detecting ADRs in Ophthalmology, but they may have methodological issues.

Innovations and breakthroughs

This is the first estimate of the frequency of ophthalmic ADRs through the use of administrative databases, and the first to apply a nationwide estimate of ophthalmic ADRs, in Portugal.

Applications

The authors suggest complementing spontaneous reporting with this database methodology to increase detection of ophthalmic ADRs.

Peer review

This is a well written article reporting the adverse effects of ophthalmic drugs. The methods are well described, and the results are easy to understand.

REFERENCES

1 **Davies EC**, Green CF, Mottram DR, Pirmohamed M. Adverse drug reactions in hospital in-patients: a pilot study. *J Clin Pharm Ther* 2006; **31**: 335-341 [PMID: 16882102]
2 **Miguel A**, Azevedo LF, Araújo M, Pereira AC. Frequency of adverse drug reactions in hospitalized patients: a systematic review and meta-analysis. *Pharmacoepidemiol Drug Saf* 2012; **21**: 1139-1154 [PMID: 22761169 DOI: 10.1002/pds.3309]
3 **Kongkaew C**, Noyce PR, Ashcroft DM. Hospital admissions associated with adverse drug reactions: a systematic review of prospective observational studies. *Ann Pharmacother* 2008; **42**: 1017-1025 [PMID: 18594048]
4 **Lazarou J**, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA* 1998; **279**: 1200-1205 [PMID: 9555760]
5 **Fraunfelder FW**, Fraunfelder FT. Scientific challenges in postmarketing surveillance of ocular adverse drug reactions. *Am J Ophthalmol* 2007; **143**: 145-149 [PMID: 17188050]
6 **World Health Organization**. Adverse Drug Reaction Monitoring. Available from: URL: http://www.who.int/medicines/areas/quality_safety/safety_efficacy/advdrugreactions/en/index.html
7 **World Health Organization**. The Importance of Pharmacovigilance - Safety Monitoring of Medicinal Products. Available from: URL: http://apps.who.int/medicinedocs/en/d/Js4893e/
8 **Naranjo CA**, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janecek E, Domecq C, Greenblatt DJ. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* 1981; **30**: 239-245 [PMID: 7249508]
9 **Davies EC**, Green CF, Mottram DR, Pirmohamed M. Adverse drug reactions in hospitals: a narrative review. *Curr Drug Saf* 2007; **2**: 79-87 [PMID: 18690953]
10 **Miguel A**, Azevedo LF, Lopes F, Freitas A, Pereira AC. Methodologies for the detection of adverse drug reactions: comparison of hospital databases, chart review and spontaneous reporting. *Pharmacoepidemiol Drug Saf* 2013; **22**: 98-102 [PMID: 23027707 DOI: 10.1002/pds.3348]
11 **Figueiras A**, Herdeiro MT, Polónia J, Gestal-Otero JJ. An educational intervention to improve physician reporting of adverse drug reactions: a cluster-randomized controlled trial. *JAMA* 2006; **296**: 1086-1093 [PMID: 16954488 DOI: 10.1001/jama.296.9.1086]
12 **Bandekar MS**, Anwikar SR, Kshirsagar NA. Quality check of spontaneous adverse drug reaction reporting forms of different countries. *Pharmacoepidemiol Drug Saf* 2010; **19**: 1181-1185 [PMID: 20845409]
13 **Pourseyed S**, Fattahi F, Pourpak Z, Gholami K, Shariatpanahi SS, Moin A, Kazemnejad A, Moin M. Adverse drug reactions in patients in an Iranian department of internal medicine. *Pharmacoepidemiol Drug Saf* 2009; **18**: 104-110 [PMID: 19101919]
14 **van der Hooft CS**, Sturkenboom MC, van Grootheest K,

Kingma HJ, Stricker BH. Adverse drug reaction-related hospitalisations: a nationwide study in The Netherlands. *Drug Saf* 2006; **29**: 161-168 [PMID: 16454543]
15 **Slee VN**. The International Classification of Diseases: ninth revision (ICD-9). *Ann Intern Med* 1978; **88**: 424-426 [PMID: 629506]
16 **Karch FE**, Lasagna L. Adverse drug reactions. A critical review. *JAMA* 1975; **234**: 1236-1241 [PMID: 1242749]
17 **Edwards IR**, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. *Lancet* 2000; **356**: 1255-1259 [PMID: 11072960]
18 **World Health Organization**. WHO Draft Guidelines for Adverse Event Reporting and Learning Systems. 2005. Available from: URL: http://www.who.int/patientsafety/events/05/Reporting_Guidelines.pdf
19 **Nebeker JR**, Barach P, Samore MH. Clarifying adverse drug events: a clinician's guide to terminology, documentation, and reporting. *Ann Intern Med* 2004; **140**: 795-801 [PMID: 15148066]
20 INFARMED - National Portuguese Authority of Drug and Health Products. Available from: URL: http://www.infarmed.pt
21 **Fong DS**, Frederick AR, Richter CU, Jakobiec FA. Adrenochrome deposit. *Arch Ophthalmol* 1993; **111**: 1142-1143 [PMID: 8352696]
22 **Azzouni F**, Abu samra K. Are phosphodiesterase type 5 inhibitors associated with vision-threatening adverse events? A critical analysis and review of the literature. *J Sex Med* 2011; **8**: 2894-2903 [PMID: 21771280]
23 **Seiber EE**. Physician code creep: evidence in Medicaid and State Employee Health Insurance billing. *Health Care Financ Rev* 2007; **28**: 83-93 [PMID: 17722753]
24 **Whitstock MT**, Pearce CM, Ridout SC, Eckermann EJ. Using clinical trial data and linked administrative health data to reduce the risk of adverse events associated with the uptake of newly released drugs by older Australians: a model process. *BMC Public Health* 2011; **11**: 361 [PMID: 21600026]

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Prevalence of hypertension in India: A meta-analysis

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Abstract

AIM: To determine the prevalence of hypertension in the urban and rural population of India.

METHODS: Relevant studies were identified through computer based and manual searches using MEDLINE/PubMed, Google scholar, EMBASE, Cochrane Library and reference lists of prevalence studies from January 2000 to June 2012. A total of 12 studies were included in the meta-analysis of hypertension in urban India and 10 studies in the analysis of hypertension in rural India after applying the inclusion and exclusion criteria. Estimates of prevalence were calculated using the random effect model for meta-analysis.

RESULTS: The electronic search using appropriate keywords identified 177 titles for prevalence of hypertension in urban India, of which 165 were excluded, and 133 titles for prevalence in rural India, of which 123 were excluded after applying the inclusion criteria. Twelve studies including 125333 subjects were analyzed to assess the prevalence of hypertension in the urban Indian population, whereas ten studies including 24800 subjects were analyzed to determine the prevalence of hypertension in the rural Indian population. The prevalence of hypertension in the urban population was estimated to be 40.8% (95%CI: 40.5%-41.0%) and that of hypertension in the rural population was 17.9% (95%CI: 17.5%-18.3%). It is evident that the prevalence of hypertension is significantly higher in the urban population of India compared to the rural.

CONCLUSION: Current evidence suggests that policies and interventions should be prioritized for reduction of hypertension in the adult Indian population, especially the urban population.

Key words: Prevalence; Hypertension; Meta-analysis; India; Urban; Rural

Core tip: A meta-analysis of prevalence studies on hypertension in India from January 2000 to June 2012 reveals a high prevalence of hypertension in the urban (40.8%) as well as rural population (17.9%). The prevalence of hypertension is markedly higher in the urban population compared to the rural population, but the prevalence in the rural population is also a matter of concern with almost every fifth individual at risk. This is indicative of the epidemiological transition, which must raise an alarm for policy makers and health care professionals. Primordial and primary prevention of hypertension can bring about a substantial reduction in cardiovascular morbidity and mortality which occurs as a consequence of hypertension.

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INTRODUCTION

Globally, the overall prevalence of hypertension or raised blood pressure in adults aged 25 and above was around 40% in 2008[1]. Worldwide, hypertension is estimated to cause 7.5 million deaths, about 12.8% of the total deaths. Hypertension accounts for 57 million disability adjusted life years (DALYS) or 3.7% of total DALYS[1]. The World Health Organization (WHO) has estimated that globally about 62% of cerebrovascular diseases and 49% of ischemic heart diseases are attributable to suboptimal blood pressure (systolic > 115 mmHg), with little variation by sex[2]. One in three adults worldwide has high blood pressure. Hypertension increases the risk of heart attack, stroke, kidney failure and many other associated co morbidities. Treating raised blood pressure and maintaining it below 140/90 mmHg is associated with a reduction in cardiovascular complications[1].

The theme for World Health Day (WHD) 2013 is "high blood pressure"[3]. The goal of WHD 2013 is to reduce heart attacks and strokes. Keeping in line with the WHO-Government of India Country Cooperation Strategy, the WHD 2013 events in India are aimed at raising the awareness amongst national policymakers, program managers and other stakeholders on the need to strengthen the Indian health system to make it competent enough to respond to hypertension and related co morbidities[3].

Hypertension is a controllable disease and it has been reported that targeted reductions in people with hypertension are expected to produce large reductions in the burden of cardiovascular disease[4]. According to the seventh report of the Joint National Committee (JNC-7) on prevention, detection, evaluation and treatment of high blood pressure, adoption of healthy lifestyles by all individuals is critical for the prevention of high blood pressure[5]. Accurate estimates of hypertension are therefore necessary to plan effective control measures.

A meta-analysis showed an increase in the prevalence of hypertension in India over the years from 1%-3% in 1950 to 10%-30.9% in 2002[6]. Another cause for concern is the epidemiological transition, as it is likely that the prevalence of risk factors, and consequently the prevalence of hypertension and cardiovascular diseases, would rise with the socioeconomic development of rural areas in India.

India accounts for 17% of the world's population, the second largest in the world, and hence it contributes largely to the statistics of any disease in the world[7]. Given the fact that hypertension is on the rise in developing countries like India, this meta-analysis was designed to consolidate the available data to find out the current prevalence of hypertension in urban and rural India.

MATERIALS AND METHODS

Search strategy

We searched MEDLINE/PubMed, Google scholar, EMBASE, Cochrane Library and reference lists of prevalence studies from January 2000 to June 2012. Internet searches used permutations of medical subject headings for prevalence studies on hypertension in India. The following keywords were looked for individually or in association: hypertension, India, prevalence, blood pressure, systolic, diastolic, mmHg. The limits included were: English for the language category and humans for the study category (Figure 1).

Selection criteria

The studies that met all of the following criteria were included in the present meta-analysis: (1) they were prevalence studies; (2) the study design was cross-sectional; (3) the age group included in the study was 20 years and above; (4) the study was conducted in the Indian population; (5) the cut-off for classification of hypertension was systolic blood pressure $\geqslant$ 140 mmHg and/or diastolic blood pressure $\geqslant$ 90 mmHg; and (6) the study contained original data. All the studies had a cross-sectional design and blood pressure measurement on a single visit was considered. Exclusion criteria: reviews, letters to editors, case series and case-control studies were not included because of insufficient data for analysis.

Statistical analysis

Data was analyzed using the statistical software Comprehensive Meta-analysis V2. The random effect model was used to calculate the estimate of the prevalence of hypertension rather than the fixed effect model. The random effect model takes into account any heterogeneity inherent in the meta-analysis.

RESULTS

Literature review

The electronic search in Pubmed, using the keywords "prevalence of hypertension in urban India", identified 177 titles, of which 165 were excluded based on review of titles, abstracts and text after applying the inclusion criteria. To avoid bias due to selection criteria and blood pressure criteria used in various studies, age, blood pressure cut-off and study design criteria were taken into consideration. The remaining 12 studies were included in the analysis. Similarly, following electronic search in Pubmed, using the keywords "prevalence of hypertension in rural India", we identified 133 titles, of which 123 were excluded based on review of titles, abstracts and text after applying the inclusion criteria. The remaining 10 were included in the analysis.

Study characteristics

In total, 12 studies were included in the meta-analysis of hypertension in urban India and 10 studies in the analysis of hypertension in rural India.

Table 1 Review of studies on the prevalence of hypertension

First author	Place	Age group (yr)	Sample size	Prevalence
In the urban Indian population				
Anand[10]	Maharashtra	30-60	1662	34.0
Gupta *et al*[11]	Rajasthan	> 20	1123	33.4
Shanthirani *et al*[12]	Tamil Nadu	> 20	1262	21.1
Gupta *et al*[18]	Maharashtra	> 35	88653	47.9
Prabhakaran *et al*[16]	Delhi	20-59	2935	30.0
Reddy *et al*[13]	Multi-centric	20-69	19973	27.7
Mohan *et al*[14]	Tamil Nadu	> 20	2350	20.0
Kaur *et al*[15]	Tamil Nadu	18-69	2262	27.2
Yadav *et al*[33]	Uttar Pradesh	> 30	1746	32.2
Midha *et al*[17]	Uttar Pradesh	> 20	400	32.8
Gupta[27]	Multi-centric	35-70	926	48.2
Chakraborty *et al*[9]	West Bengal	18-60	433	17.6
In the rural Indian population				
Kusuma *et al*[31]	Andhra Pradesh	> 20	1316	21.0
Hazarika *et al*[30]	Assam	> 30	3180	33.3
Midha *et al*[17]	Uttar Pradesh	> 20	400	14.5
Todkar *et al*[19]	Maharashtra	> 20	1297	7.2
Bhardwaj *et al*[24]	Himachal Pradesh	> 18	1092	35.9
Kinra *et al*[32]	Multi-centric	20-69	1983	20.0
Rajasekar *et al*[20]	Tamil Nadu	> 30	1905	19.1
Kadu *et al*[21]	Maharashtra	> 18	2196	12.8
Bansal *et al*[22]	Uttarakhand	> 15	968	32.3
Kaur *et al*[23]	Tamil Nadu	25-64	10463	21.4

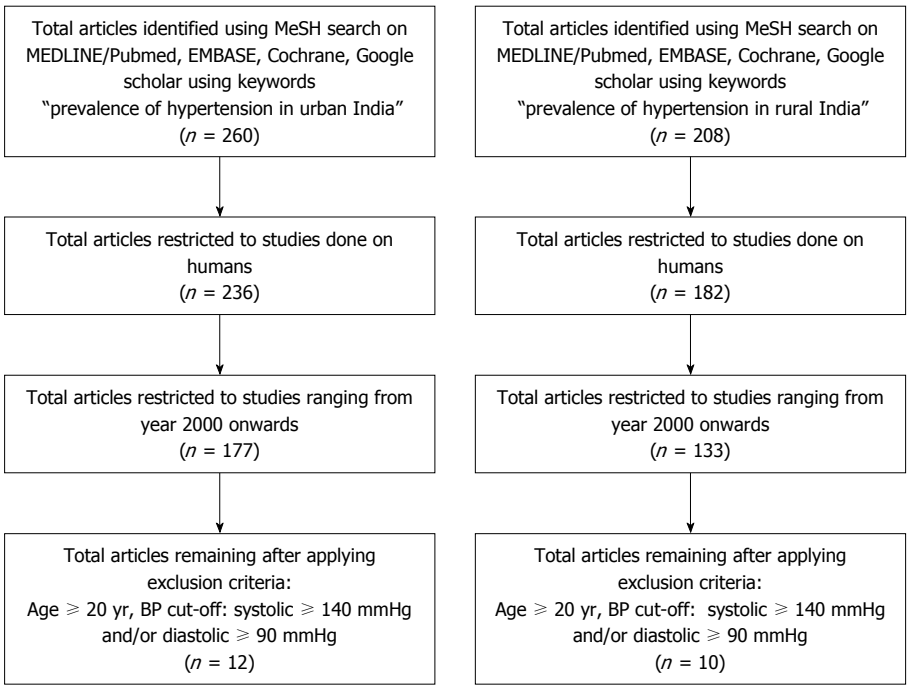

Figure 1 Flow diagram of selection process. Course of systematic literature review on prevalence of hypertension in urban and rural India. BP: Blood pressure.

Meta-analysis

After analysis of 125333 subjects from twelve studies, the prevalence of hypertension in the urban Indian population was found to be 40.8% (95%CI: 40.5%-41.0%) (Table 1). Ten studies including 24800 subjects were analysed and the prevalence of hypertension in the rural population was estimated to be 17.9% (95%CI: 17.5%-18.3%) (Table 1). Figure 2 shows the meta-analysis of prevalence of hypertension in the urban and rural Indian population respectively. The overall prevalence rate is represented by the random effect size which was estimated to be 40.8% in urban and 17.9% in the rural population.

DISCUSSION

In the present meta-analysis, the prevalence of hypertension was estimated to be 40.8% in urban and 17.9% in

A

Meta Analysis

Study name	Statistics for each study						
	Point estimate	SE	Variance	Lower limit	Upper limit	*Z*-value	*P*-value
Anand[10]	34.00	1.160	1.34	31.72	36.27	29.310	0.000
Gupta *et al*[11]	33.40	1.410	1.98	30.63	36.16	23.688	0.000
Shanthirani *et al*[12]	21.10	1.150	1.32	18.84	23.35	18.348	0.000
Gupta *et al*[18]	47.90	0.170	0.02	47.56	48.23	281.765	0.000
Prabhakaran *et al*[16]	30.00	0.850	0.72	28.33	31.66	35.294	0.000
Reddy *et al*[13]	27.20	0.310	0.09	26.59	27.80	87.742	0.000
Mohan *et al*[14]	20.00	0.830	0.68	18.37	21.62	24.096	0.000
Kaur *et al*[15]	27.20	0.940	0.88	25.35	29.04	28.936	0.000
Yadav *et al*[33]	32.20	1.120	1.25	30.00	34.39	28.750	0.000
Midha *et al*[17]	32.80	2.350	2.40	28.10	37.49	28.725	0.000
Gupta[27]	48.20	1.640	2.69	44.98	51.41	29.390	0.000
Chakraborty *et al*[9]	17.60	1.830	3.34	14.01	21.18	9.617	0.000
Random effect size	40.76	0.137	0.01	40.49	41.02	297.873	0.000

B

Meta Analysis

Study name	Statistics for each study						
	Point estimate	SE	Variance	Lower limit	Upper limit	*Z*-value	*P*-value
Kusuma *et al*[31]	21.00	1.100	1.21	18.84	23.15	19.091	0.000
Hazarika *et al*[30]	33.30	0.800	0.64	31.73	34.86	41.625	0.000
Midha *et al*[17]	14.50	1.500	0.25	11.10	17.90	18.600	0.000
Todkar *et al*[19]	7.20	0.700	0.49	5.82	8.57	10.286	0.000
Bhardwaj *et al*[24]	35.90	1.500	2.25	32.96	38.84	23.933	0.000
Kinra *et al*[32]	20.00	0.900	0.81	18.23	21.76	22.222	0.000
Rajasekar *et al*[20]	19.10	0.900	0.81	17.33	20.86	21.222	0.000
Kadu *et al*[21]	12.75	0.700	0.49	11.37	14.12	18.214	0.000
Bansal *et al*[22]	32.30	1.500	2.25	29.36	35.24	21.533	0.000
Kaur *et al*[23]	21.40	0.400	0.16	20.61	22.18	53.500	0.000
Random effect size	17.91	0.223	0.05	17.47	18.34	80.259	0.000

Figure 2 The overall prevalence rate is represented by the random effect size. A: Meta-analysis of prevalence of hypertension in the urban Indian population; B: Meta-analysis of prevalence of hypertension in the rural Indian population.

the rural Indian population. Gupta *et al*[8] reported the highest prevalence of hypertension (48.2%) in a recent multi-centric study, conducted in the urban population of India. However, Chakraborty *et al*[9] observed a lower prevalence of hypertension (17.6%) possibly because of the lower age group (18-60 years) and lower socioeconomic strata (slum dwellers) included in the study.

Anand[10] (34.0%), Gupta *et al*[11] (33.4%) and Shanthirani *et al*[12] (21.1%) in the early 2000s, and Reddy *et al*[13] (27.7%), Mohan *et al*[14] (20.0%), Kaur *et al*[15] (27.2%), Prabhakaran *et al*[16] (30.0%) and Midha *et al*[17] (32.8%) in the mid 2000s have observed that the prevalence of hypertension ranged between 20%-40% throughout the decade. However, no significant trend has been observed in the prevalence of hypertension among the studies conducted down the years. The findings of Gupta *et al*[18] reveal a prevalence of 47.9% from Mumbai, Maharashtra, probably because of the stressful lifestyle of subjects in a metropolitan city. The result of the meta-analysis summarizes all these findings and shows the prevalence of hypertension as 40.8% in the urban population of India.

In the rural population, the lowest prevalence (7.2%) was observed by Todkar *et al*[19] in Maharashtra. Midha *et al*[17] (14.5%), Rajasekar *et al*[20] (19.1%) and Kadu *et al*[21] (12.8%) have reported a lower prevalence compared to Bansal *et al*[22] (32.3%) and Kaur *et al*[23] (21.4%). Despite the lack of an obvious trend, the prevalence of hypertension in the rural population is rising swiftly to match up to the urban rates. Bhardwaj *et al*[24] reported the highest prevalence of hypertension (35.9%) in the rural population of Himachal Pradesh. The meta-analysis revealed a prevalence rate of 17.9% among the rural population.

From the available data, it is obvious that the prevalence of hypertension is higher in the urban population of India compared to the rural. No consistent trends are visible with respect to regional variations. In rural populations, the prevalence of hypertension is higher in Himachal Pradesh, while in urban studies prevalence rate is higher in Maharashtra[18,24]. The prevalence rate of hypertension was amongst the highest in metropolitan cities like Mumbai[18]. Moreover, the prevalence of hypertension in rural populations is steadily increasing and is approaching the rates of the urban population. Several studies have reported that there are significant urban-rural differences in metabolic cardiovascular risk factors[25]. Prevalence of smoking is greater in rural men while all other risk factors, such as sedentary lifestyle, obesity, central obesity, hypercholesterolemia, diabetes and the metabolic syndrome, are more common in urban men and women[26]. Most studies on urban-rural differences in cardiovascular risk factors from Haryana, Delhi, Rajasthan and Tamil Nadu have reported greater prevalence of

multiple CVD risk factors in the urban population[27]. As a result, cardiovascular diseases are epidemic in the urban regions of low income countries such as India. However, greater prevalence of cardiovascular risk factors in urban areas in India is in contrast to high income countries where the CVD risk factors are equal in urban and rural areas[28]. Similarly, it has been observed that in the more developed states of India, such as Kerala, the rural-urban differences in cardiometabolic risk factors have largely disappeared and the risk factors are equal or slightly greater in the rural population[29]. Hazarika *et al*[30] reported that even in the rural population of Assam, body mass index and waist-hip ratio were significant risk factors of hypertension. Kusuma *et al*[31] confirmed the hypothesis that acculturation/modernization may elevate the risk of hypertension and that prevalence is generally low among traditional population groups. Kinra *et al*[32] suggested that a nutrition transition (coexistence of over-nutrition and under-nutrition) may have progressed to some parts of rural India. He observed that obesity, dyslipidemia, diabetes and hypertension were more prevalent in higher socioeconomic groups in the rural areas. This epidemiological transition is a cause for serious concern as it is likely that the prevalence of risk factors, and thereby the prevalence of hypertension and cardiovascular diseases, would rise with the socioeconomic development of rural areas.

Yadav *et al*[33] observed that there was a high prevalence of cardiovascular risk factors in the general population [central obesity (86.7%), elevated LDL cholesterol (22.8%), abnormal glucose tolerance (41.6%) and smoking (20.3% of males)]. Two or more of the cardiovascular risk factors were present in a higher proportion of hypertensives (66%, OR = 3.0, $P < 0.0001$) and pre-hypertensives, (56%, OR = 2.0, $P < 0.0001$) compared to normotensive subjects (39%). The current rate of hypertension in the urban areas and the rising trend in the rural population is a warning to institute lifestyle changes in the community in order to put a halt to the increasing rates.

Challenge ahead

The high prevalence of hypertension in the urban and rural population in India presents a formidable challenge to the Indian health system. In countries like India, the out-of-pocket expenditures incurred for non-communicable diseases (NCDs) like hypertension are high, which hits the impoverished households the most. Medicines for these chronic diseases account for a large portion of expenditure. Therefore, population based prevention strategies have a high impact and are cost-effective as these target lifestyle change. Interventions utilizing the power of public policies for reducing salt, fat, sugar and alcohol intake through regulatory and consumer education approaches; increasing physical activity through sound urban planning and creation of activity-promoting environments; increasing fruit and vegetable intake through appropriate agricultural and pricing mechanisms; and implementing comprehensive tobacco control have the potential to prevent a large proportion of disease events in the whole population[34].

Hypertension is easily diagnosable and treatable with lifestyle modifications and effective medicines. Furthermore, hypertension control provides an entry point to deal with other NCDs as any intervention will help to concomitantly address other NCDs as well. This has been taken into cognizance in the newly launched National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS), which has hypertension and diabetes as the main focus areas[34]. National strategies will focus on prevention and health promotion as the key to reduce disease burden. Health education programs that promote exercise, weight reduction, early diagnosis and screening are some of the key interventions that will be promoted at various levels of heath facilities[35]. Under the NPCDCS, the strategy for early diagnosis of chronic NCDs will consist of opportunistic screening of persons above the age of 30 years at the point of primary contact with any health care facility[35]. The NCD clinics mandated under NPCDCS could be leveraged to facilitate guidelines based hypertension management with emphasis on generic drugs and those recommended by the Indian Public Health Standards[34].

In conclusion, the high prevalence of hypertension in the urban population and a rising prevalence in the rural population must raise an alarm for policy makers and health care professionals as this is an area where primordial and primary prevention measures can bring about a substantial reduction in cardiovascular morbidity and mortality in the future.

Limitations

Given the limited amount of data available on the prevalence of hypertension in India, studies conducted in subjects with different bio-social characteristics have been included in the meta-analysis. Nevertheless, the pooled estimate does provide an overview of the magnitude of the problem of hypertension in the Indian population.

COMMENTS

Background

The overall prevalence of hypertension in adults aged 25 and over was around 40% in the world in 2008 and a meta-analysis estimated a prevalence of 10-30.9% in India in 2002. This shift in epidemiological profile presents a unique challenge to India's health system as rates of cardiovascular and metabolic disease like hypertension and diabetes, obesity and cancer rise, tuberculosis, diarrheal disease and water borne illnesses remain widespread. According to a 2012 World Health Organization report, non communicable diseases are responsible for two-thirds of the total morbidity burden and about 53% of total deaths in India. Hypertension provides an entry point to other non-communicable diseases. Therefore, a precise estimate of the prevalence of hypertension in the urban and rural population of the India is required to assess the magnitude of the problem that has to be addressed.

Research frontiers

Very few studies are available on the prevalence of hypertension in India. A consolidated estimate of hypertension from various studies conducted in different regions of the country can aid the development of preventive strategies. The difference in prevalence between the urban and rural population has also

been studied to provide an insight to the kind of preventive and promotive services required.

Innovations and breakthroughs

It is possible that an insight into the magnitude of the problem of hypertension can help in shaping the preventive programs and policies specific for the rural and urban population.

Applications

Very few multi-centric studies are available in India; therefore, the main application of this meta-analysis is to consolidate the available data to determine the burden of hypertension in country.

Terminology

A meta-analysis integrates the quantitative findings from separate but similar studies and provides a numerical estimate of the overall effect of interest. Different weights are assigned to the different studies for calculating the summary or pooled effect. The weighting is related with the inverse of the standard error (and therefore indirectly to the sample size) reported in the studies. Studies with smaller standard error and larger sample size are given more weight in the calculation of the pooled effect size. The meta-analysis table lists the prevalence of hypertension (expressed as a percentage), with their 95%CI found in the individual studies included in the meta-analysis. The pooled proportion (prevalence) with 95%CI is given for the random effects model. The random effects model will tend to give a more conservative estimate (*i.e.*, with wider confidence interval), but the results are more valid as they take into account any inherent heterogeneity. Under the random effects model, the true effects in the studies are assumed to vary between studies and the summary effect is the weighted average of the effects reported in the different studies.

Peer review

This manuscript is a meta-analysis on the prevalence of hypertension in India. Its results have provided evidence for policies and interventions for hypertension. It is well written.

REFERENCES

1 **World Health Organization**. Global Health Repository. Available from: URL: http://www.who.int/gho/ncd/risk_factors/blood_pressure_prevalence_text/en/index.html. Last accessed April 24, 2013

2 World Health Report-2002. Reducing Risks, Promoting Healthy Life. Chapter 4, p-12. Available from: URL: http://www.who.int/whr/2002/en/whr02_ch4.pdf. Last accessed April 24, 2013

3 Available from: URL: http://www.who.int/world-health-day/en/. Last accessed April 24, 2013

4 **Rodgers A**, Lawes C, MacMahon S. Reducing the global burden of blood pressure-related cardiovascular disease. *J Hypertens Suppl* 2000; **18**: S3-S6 [PMID: 10939783]

5 **Chobanian AV**, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; **289**: 2560-2572 [PMID: 12748199]

6 **Padmavati S**. Prevention of heart disease in India in the 21st century: need for a concerted effort. *Indian Heart J* 2002; **54**: 99-102 [PMID: 11999100]

7 World Development Report-2006. Equity and Development. A co-publication of the World Bank and Oxford University Press

8 **Gupta R**, Pandey RM, Misra A, Agrawal A, Misra P, Dey S, Rao S, Menon VU, Kamalamma N, Vasantha Devi KP, Revathi K, Vikram NK, Sharma V, Guptha S. High prevalence and low awareness, treatment and control of hypertension in Asian Indian women. *J Hum Hypertens* 2012; **26**: 585-593 [PMID: 21881598 DOI: 10.1038/jhh.2011.79]

9 **Chakraborty R**, Bose K, Kozieł S. Waist circumference in determining obesity and hypertension among 18-60 years old Bengalee Hindu male slum dwellers in Eastern India. *Ann Hum Biol* 2011; **38**: 669-675 [PMID: 21838593]

10 **Anand MP**. Prevalence of hypertension amongst Mumbai executives. *J Assoc Physicians India* 2000; **48**: 1200-1201 [PMID: 11280229]

11 **Gupta R**, Gupta VP, Sarna M, Bhatnagar S, Thanvi J, Sharma V, Singh AK, Gupta JB, Kaul V. Prevalence of coronary heart disease and risk factors in an urban Indian population: Jaipur Heart Watch-2. *Indian Heart J* 2002; **54**: 59-66 [PMID: 11999090]

12 **Shanthirani CS**, Pradeepa R, Deepa R, Premalatha G, Saroja R, Mohan V. Prevalence and risk factors of hypertension in a selected South Indian population--the Chennai Urban Population Study. *J Assoc Physicians India* 2003; **51**: 20-27 [PMID: 12693449]

13 **Reddy KS**, Prabhakaran D, Chaturvedi V, Jeemon P, Thankappan KR, Ramakrishnan L, Mohan BV, Pandav CS, Ahmed FU, Joshi PP, Meera R, Amin RB, Ahuja RC, Das MS, Jaison TM. Methods for establishing a surveillance system for cardiovascular diseases in Indian industrial populations. *Bull World Health Organ* 2006; **84**: 461-469 [PMID: 16799730]

14 **Mohan V**, Deepa M, Farooq S, Datta M, Deepa R. Prevalence, awareness and control of hypertension in Chennai--The Chennai Urban Rural Epidemiology Study (CURES-52). *J Assoc Physicians India* 2007; **55**: 326-332 [PMID: 17844691]

15 **Kaur P**, Rao TV, Sankarasubbaiyan S, Narayanan AM, Ezhil R, Rao SR, Gupte MD. Prevalence and distribution of cardiovascular risk factors in an urban industrial population in south India: a cross-sectional study. *J Assoc Physicians India* 2007; **55**: 771-776 [PMID: 18290552]

16 **Prabhakaran D**, Shah P, Chaturvedi V, Ramakrishnan L, Manhapra A, Reddy KS. Cardiovascular risk factor prevalence among men in a large industry of northern India. *Natl Med J India* 2005; **18**: 59-65 [PMID: 15981439]

17 **Midha T**, Idris MZ, Saran RK, Srivastav AK, Singh SK. Prevalence and determinants of hypertension in the urban and rural population of a north Indian district. *East Afr J Public Health* 2009; **6**: 268-273 [PMID: 20803917]

18 **Gupta PC**, Gupta R, Pednekar MS. Hypertension prevalence and blood pressure trends in 88 653 subjects in Mumbai, India. *J Hum Hypertens* 2004; **18**: 907-910 [PMID: 15306829 DOI: 10.1038/sj.jhh.1001763]

19 **Todkar SS**, Gujarathi VV, Tapare VS. Period prevalence and sociodemographic factors of hypertension in rural maharashtra: a cross-sectional study. *Indian J Community Med* 2009; **34**: 183-187 [PMID: 20049292 DOI: 10.4103/0970-0218.55269]

20 **Rajasekar VD**, Krishnagopal L, Mittal A, Singh Z, Purty AJ, Binu VS, Iillayabharathi V. Prevalence and risk factors for hypertension in a rural area of Tamil Nadu, South India. *Indian J Med Spec* 2012; **3**: 12-17 [DOI: 10.7713/ijms.2012.0004]

21 **Kadu AV**, Mane SS, Lakde RN, Vedpathak VL, Gaikwad AE, Choudhari SG. Prevalence of Hypertension in the Rural Community of Central Maharashtra, India. *Int J Med Public Health* 2012; **2**; 39-45

22 **Bansal SK**, Saxena V, Kandpal SD, Gray WK, Walker RW, Goel D. The prevalence of hypertension and hypertension risk factors in a rural Indian community: A prospective door-to-door study. *J Cardiovasc Dis Res* 2012; **3**: 117-123 [PMID: 22629029 DOI: 10.4103/0975-3583.95365]

23 **Kaur P**, Rao SR, Radhakrishnan E, Rajasekar D, Gupte MD. Prevalence, awareness, treatment, control and risk factors for hypertension in a rural population in South India. *Int J Public Health* 2012; **57**: 87-94 [PMID: 21947549]

24 **Bhardwaj R**, Kandori A, Marwah R, Vaidya P, Singh B, Dhiman P, Sharma A. Prevalence, awareness and control of hypertension in rural communities of Himachal Pradesh. *J Assoc Physicians India* 2010; **58**: 423-424, 429 [PMID: 21121207]

25 **Gupta R**, Joshi P, Mohan V, Reddy KS, Yusuf S. Epidemiology and causation of coronary heart disease and stroke in India. *Heart* 2008; **94**: 16-26 [PMID: 18083949 DOI: 10.1136/hrt.2007.132951]

26 **Gupta R**, Gupta VP. Urban-rural differences in coronary risk factors do not fully explain greater urban coronary heart dis-

ease prevalence. *J Assoc Physicians India* 1997; **45**: 683-686
27 **Gupta R**. Epidemiology and regional variations in cardiovascular disease and risk factors in India. *J Prev Cardiol* 2011; **1**: 7-15
28 **Stuckler D**. Population causes and consequences of leading chronic diseases: a comparative analysis of prevailing explanations. *Milbank Q* 2008; **86**: 273-326 [PMID: 18522614 DOI: 10.1111/j.1468-0009.2008.00522.x]
29 **Thankappan KR**, Shah B, Mathur P, Sarma PS, Srinivas G, Mini GK, Daivadanam M, Soman B, Vasan RS. Risk factor profile for chronic non-communicable diseases: results of a community-based study in Kerala, India. *Indian J Med Res* 2010; **131**: 53-63 [PMID: 20167974]
30 **Hazarika NC**, Narain K, Biswas D, Kalita HC, Mahanta J. Hypertension in the native rural population of Assam. *Natl Med J India* 2004; **17**: 300-304 [PMID: 15736549]
31 **Kusuma YS**, Babu BV, Naidu JM. Prevalence of hypertension in some cross-cultural populations of Visakhapatnam district, South India. *Ethn Dis* 2004; **14**: 250-259 [PMID: 15132211]
32 **Kinra S**, Bowen LJ, Lyngdoh T, Prabhakaran D, Reddy KS, Ramakrishnan L, Gupta R, Bharathi AV, Vaz M, Kurpad AV, Smith GD, Ben-Shlomo Y, Ebrahim S. Sociodemographic patterning of non-communicable disease risk factors in rural India: a cross sectional study. *BMJ* 2010; **341**: c4974 [PMID: 20876148 DOI: 10.1136/bmj.c4974]
33 **Yadav S**, Boddula R, Genitta G, Bhatia V, Bansal B, Kongara S, Julka S, Kumar A, Singh HK, Ramesh V, Bhatia E. Prevalence & risk factors of pre-hypertension & hypertension in an affluent north Indian population. *Indian J Med Res* 2008; **128**: 712-720 [PMID: 19246794]
34 **Mohan S**, Campbell N, Chockalingam A. Time to effectively address hypertension in India. *Indian J Med Res* 2013; **137**: 627-631 [PMID: 23703328]
35 Operational guidelines. National programme for prevention and control of cancer, diabetes, cardiovascular diseases and stroke (NPCDCS). Directorate General of Health Services. Ministry of Health and Family Welfare. Government of India. Available from: URL: http://health.bih.nic.in/Docs/Guidelines/Guidelines-NPCDCS.pdf. Last accessed August 2013

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META-ANALYSIS

Subclinical hypothyroidism and the metabolic syndrome: A meta-analysis of cross-sectional studies

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Abstract

AIM: To determine the relationship between subclinical hypothyroidism (SCH) and the metabolic syndrome (MS).

METHODS: We performed a systematic search of databases [MEDLINE (July 1950 to July 2012), EMBASE (July 1966 to July 2012)] and the references of identified studies. Completely published cross-sectional studies of a general population involving SCH and the MS were included. The pooled odds ratio and weighted mean difference (WMD) for the outcomes were calculated using random-effects models.

RESULTS: Six cross-sectional studies with 19546 participants were included. In total, 398 of 1324 participants (30.06%) in the SCH group had the MS compared with 4975 of 18222 participants (27.30%) in the euthyroid group [OR = 1.20; 95%CI: 1.05-1.36; P = 0.004; χ^2 = 2.53 (P = 0.773); I^2 = 0%]. Further analysis of the components of the MS showed that SCH was associated with increased body mass index (WMD, 0.32 kg/m^2; 95%CI: 0.04-0.61; P = 0.026), systolic blood pressure (WMD, 2.62 mmHg; 95%CI: 1.35-3.89; P < 0.001) and triglyceride (WMD, 0.25 mmol/L; 95%CI: 0.23-0.28; P < 0.001).

CONCLUSION: Based on the cross-sectional data, SCH may be associated with an increased risk of the MS, which could be attributed to the increased risk of metabolic components.

Key words: Subclinical hypothyroidism; Metabolic syndrome; Meta-analysis

Core tip: A recent meta-analysis of individual data concluded that subclinical hypothyroidism (SCH) is associated with an increased risk of coronary heart disease (CHD) and CHD mortality. Meanwhile, it has been well recognized that the metabolic syndrome (MS) is associated with increased cardiovascular events and all-cause mortality. Our meta-analysis of cross-sectional data demonstrated that SCH may be associated with an increased risk of the MS, which may explain the relationship between SCH and increased risk of CHD.

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INTRODUCTION

Subclinical thyroid disease is defined biochemically and subclinical hypothyroidism (SCH) occurs when serum thyroid-stimulating hormone (TSH) concentrations are raised and serum thyroid hormone concentrations are normal[1].

Over the past several decades, a plethora of publications has established that SCH is associated with cardiovascular disease and a meta-analysis of individual data by Rodondi *et al*[2] concluded that SCH is associated with an increased risk of coronary heart disease (CHD) and CHD mortality in those with higher TSH levels, particularly in those with a TSH concentration of 10 mIU/L or greater.

The metabolic syndrome (MS) is a cluster of multiple cardiovascular risk factors, including central obesity, glucose intolerance, diabetes, dyslipidemia and elevated blood pressure[3]. It has been well recognized that the MS is associated with increased cardiovascular events and all-cause mortality[4]. Recently, several cross-sectional studies have investigated a potential relationship between SCH and the MS, with conflicting findings[5-10]. To clarify this issue, we performed a meta-analysis of the published cross-sectional data in a general population to determine the association between SCH and the MS.

MATERIALS AND METHODS

We designed a protocol that detailed the objective of our analysis, the criteria for study inclusion/exclusion, the assessment of study quality, the primary outcome and the statistical methods in accordance with the meta-analysis of observational studies in epidemiology[11] and PRISMA statements[12].

Data sources and searches

We conducted a search of MEDLINE (1950 to July 2012) and EMBASE (1966 to July 2012) *via* EMBASE.com to identify all cross-sectional studies of a general population involving SCH and the MS [search strategy: (1) "hypothyroidism"/exp OR hypothyroidism; (2) "thyroid dysfunction"/exp OR thyroid dysfunction; (3) "thyroid disease"/exp OR thyroid disease; (4) "thyrotropin"/exp OR thyrotropin; (5) "thyroid stimulation hormone"/exp OR thyroid stimulation hormone; (6) "MS x"/exp OR MS x; (7) "MS"/exp OR MS; (8) 1 OR 2 OR 3 OR 4 OR 5; (9) 6 OR 7; (10) 8 AND 9], as well as a search of the Cochrane Database for Systemic Reviews. In addition, we performed a manual search of the literature using the references of original manuscripts, reviews and meta-analyses.

Study selection

Two reviewers (Ye YC and Xie HZ) independently determined the study eligibility. Disagreements were resolved by consensus. The eligibility criteria for study inclusion were: (1) cross-sectional studies; (2) studies of a general population; (3) studies that reported the numbers of participants with the MS from among subjects with SCH and euthyroid; and (4) completely published studies (exclusive of unpublished material and abstracts). No language restriction was imposed. The κ value between two reviewers (Ye YC and Xie HZ) was 0.97 for the first screen (based on title and abstract) and 1.0 for the full-text screen.

Data extraction

Data extraction was carried out independently by two authors in duplicate (Ye YC and Xie HZ). Disagreements were resolved by discussion between the two reviewing authors. From each included study, information was extracted on: (1) the characteristics of the study population; (2) the criteria for the MS; (3) TSH reference; (4) the numbers of participants with the MS in SCH and euthyroid groups; and (5) the components of the MS in SCH and euthyroid groups [including waist circumference, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose, triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C)].

Risk of bias

Risk of bias was described and judged in following domains[13]: (1) Define the source of information (survey, record review); (2) List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications; (3) Indicate time period used for identifying individuals; (4) Indicate whether or not subjects were consecutively enrolled if not population-based; (5) Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants; (6) Describe any assessments undertaken for quality assurance purposes (*e.g.*, test/retest of primary outcome measurements); (7) Explain any individual exclusions from analysis; (8) Describe how confounding was assessed and/or controlled; (9) If applicable, explain how missing data were handled in the analysis; and (10) Summarize individual response rates and completeness of data collection. The judgments were assessed independently by two authors (Ye YC and Xie HZ) based on the published report and protocols of the studies. Disagreements were resolved by consensus. The judgments involved the answers "yes" (indicating a low risk of bias), "no" (indicating a high risk of bias), and "unclear" (if risk of bias is unknown or if an entry is not relevant to the study).

Statistical analysis

The primary outcome was the unadjusted OR for the MS between the SCH and the euthyroid group. The secondary outcomes were the weighted mean difference (WMD) of the components of the MS between the SCH and the euthyroid group. The pooled unadjusted ORs and WMD for the outcomes were calculated using fixed effects models since there may be potential heterogeneity among the studies. The heterogeneity between the results of different studies was examined using χ^2 tests for significance (a P-value < 0.10 was considered to be statistically significant) and the inconsistence was examined using I^2 tests. Subgroup analysis was used to explore the potential race heterogeneity (Chinese population and non Chinese population). Sensitivity analysis was used to explore the degree to which the main findings of our meta-analysis were affected by individual studies. Publication

Table 1 Characteristics of included studies

Studies	Age (yr)	Male (%)	MS criteria	Sample size	Definition of SCH	Euthyroid			SCH			Inclusion criteria	Exclusion criteria
						No.	MS	mTSH	No.	MS	mTSH		
Hergenç *et al*[5]	51.99	46.70	NCEP /ATPⅢ	488	TSH > 4.2 μU/mL	465	193	1.12	23	10	7.54	Age > 34 yr	NA
Garduño-Garcia Jde *et al*[6]	42.3	48.80	NCEP /ATPⅢ	3033	TSH: 4.5-10 mIU/L FT4: 10-25 pmol/L	2771	876	NA	262	84	NA	Aged 18-70 yr	Thyroid disease, diabetes, cardiovascular disease, cerebral vascular disease, amputations, pregnancy, corticosteroid use, active liver disease, and renal dysfunction
Lai *et al*[7]	NA	NA	NCEP /ATPⅢ	6254	TSH > 5.0 μU /mL Normal FT4	6123	1871	NA	131	43	8.5	Age > 65 yr	Taking medications for control of glucose, blood pressure, dyslipidemia, and thyroid function
Lai *et al*[8]	45.1	NA	China Diabetes Society	1385	TSH > 4.8 mIU/L Normal FT3/FT4	1283	238	NA	102	25	NA	Aged 18-85 yr	History of thyroid disease; Taking medication such as thyroxine/antithyroid drugs, glucocorticoid, antiepileptic and contraceptive drugs; Pregnant/within the first year of postpartum period; Overt hypothyroidism/ hyperthyroidism
Liu *et al*[9]	48.9	38.20	International Diabetes Federation	6339	TSH > 4.5 mIU/L Normal FT3/FT4	5801	1236	2	538	138	6.5	Aged 20-88 yr	Pregnant; Severe renal, liver or heart failure, or abdominal ascites; Taking medicines influencing thyroidal function
Waring *et al*[10]	73.6	47	NCEP /ATPⅢ	2047	TSH > 0.35 mIU/L	1779	561	NA	268	98	NA	Aged 70-79 yr	Diabetes; Taking medication such as amiodarone, lithium and antithyroid medications

NCEP/ATPⅢ: National Cholesterol Education Program/Adult Treatment Panel Ⅲ; TSH: Thyroid-stimulating hormone; MS: Metabolic syndrome; SCH: Subclinical hypothyroidism; FT4: Free thyroxine; FT3: Free triiodothyronine; NA: Not available; mTSH: mean/median thyroid-stimulating hormone level.

bias was assessed by the Begg's funnel plot and the Egger weighted regression statistic, with a value of $P < 0.10$ indicating significant publication bias among the included studies[14,15]. All statistical analyses were performed using STATA 11.0 (STATA, TX, United States).

RESULTS

Study identification

In total, 515 studies were retrieved from the initial search and 17 studies were reviewed in full text. Six cross-sectional studies investigating a total of 19546 participants were included in the meta-analysis (Figure 1).

Study characteristics

The analysis included a total of 1324 SCH and 18222 euthyroid is imparticipants. In the individual studies, the sample size ranged from 488 to 6339. The reported mean age of the participants ranged from 42.3 to 73.6 years. Each study included both males and females. Table 1 presents a summary of the characteristics of the study population, the criteria for the MS, the TSH reference and the prevalence of the MS in SCH and euthyroid groups. Of the six studies included, four provided data on the components of the MS in SCH and euthyroid groups (Table 2). The results of quality assessment of included studies (risk of bias) were summarized and are presented in Figure 2.

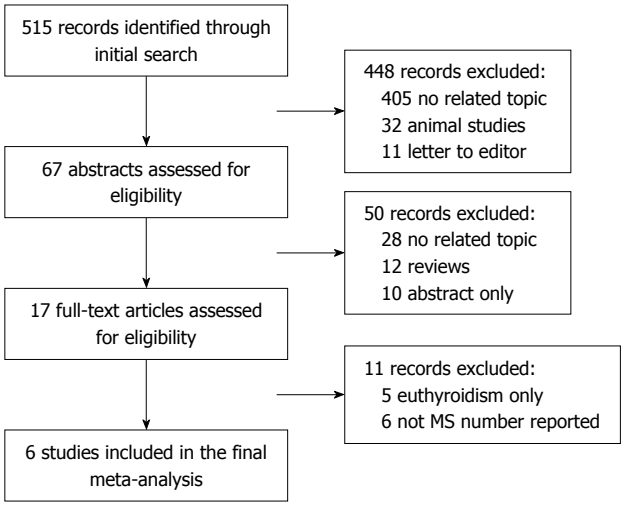

Figure 1 Flowchart of study selection. MS: Metabolic syndrome.

Data analysis of outcomes

In total, 4975 of the 18222 participants (27.30%) in the euthyroid group fulfilled the criteria for the MS, compared to 398 of the 1324 participants (30.06%) in the

Table 2 Components of the metabolic syndrome in each study

Studies	Thyroid function	*n*	Waist (cm)	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	FBG (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)
Hergenç *et al*[5]	Euthyroid	465	95.4 ± 10.5	29.3 ± 4.5	126.5 ± 20.6	80.9 ± 10.9	5.77 ± 2.30	1.92 ± 1.24	1.14 ± 0.32
	SCH	23	93.0 ± 10.5	29.0 ± 4.2	121.6 ± 23.8	78.8 ± 13.2	5.66 ± 1.51	1.81 ± 1.11	1.16 ± 0.30
Garduño-Garcia	Euthyroid	2771	94.21 ± 11.18	28.69 ± 4.5	115 ± 15.12	77.29 ± 10.68	4.90 ± 1.16	2.29 ± 1.73	1.10 ± 0.29
Jde *et al*[6]	SCH	262	94.06 ± 11.18	28.98 ± 5.1	118 ± 16.23	78.22 ± 10.87	4.91 ± 0.973	2.59 ± 4.03	1.13 ± 0.29
Lai *et al*[8]	Euthyroid	1283	80.8 ± 10.3	24.3 ± 3.6	123 ± 18	79 ± 11	5.22 ± 0.03	1.47 ± 0.03	1.33 ± 0.37
	SCH	102	80.7 ± 9.6	24.5 ± 3.3	122 ± 19	77 ± 11	5.12 ± 0.11	1.73 ± 0.12	1.26 ± 0.27
Liu *et al*[9]	Euthyroid	5801	80.4 ± 11.1	24.4 ± 6.6	126.8 ± 19.2	81.9 ± 11.3	5.2 ± 1.7	1.5 ± 1.6	1.3 ± 0.4
	SCH	538	81.1 ± 12.4	24.8 ± 3.9	130.2 ± 20.8	83.4 ± 12.0	5.3 ± 1.5	1.6 ± 1.7	1.3 ± 0.6

SCH: Subclinical hypothyroidism; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol.

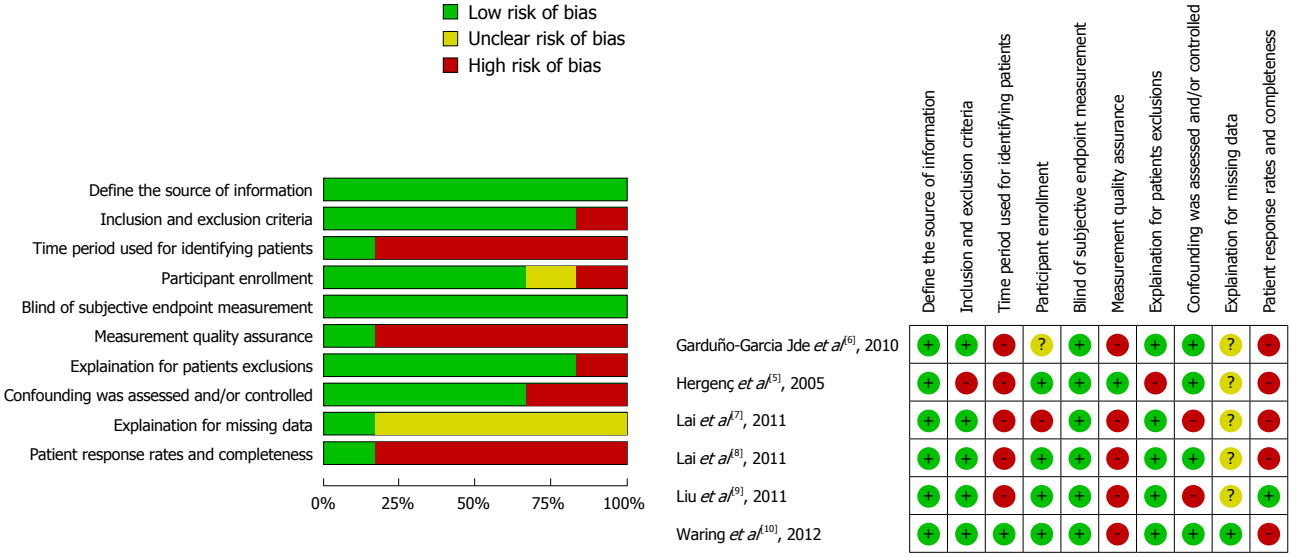

Figure 2 Risk of bias assessment.

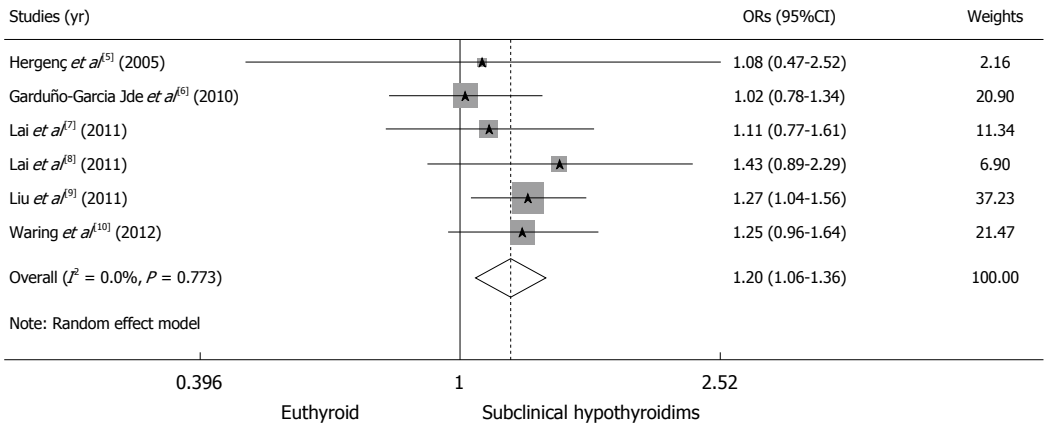

Figure 3 Subclinical hypothyroidism *vs* euthyroid on pooled ORs for the metabolic syndrome.

SCH group. The pooled risk of the MS is significantly higher in the SCH group than in the euthyroid group (OR, 1.20; 95%CI: 1.05-1.36; $P = 0.004$). We obtained a χ^2 value of 2.53 ($P = 0.773$) and an I^2 value of 0%, indicating the absence of heterogeneity among the studies analyzed (Figure 3).

Further meta-analysis of the components of the MS showed that SCH was associated with increased BMI (WMD, 0.32 kg/m^2; 95%CI: 0.04-0.61; $P = 0.026$), SBP (WMD, 2.62 mmHg; 95%CI: 1.35-3.89; $P < 0.001$) and TG (WMD, 0.25 mmol/L; 95%CI: 0.23-0.28; $P < 0.001$). The rest of the components, including waist circumfer-

Table 3 Sensitivity analysis of the effect of exclusion of individual studies on pooled ORs with 95%CI

Studies excluded	Pooled ORs of remaining studies (95%CI)	*P* for OR	I^2 (%)	χ^2	*P* for χ^2
Hergenç *et al*[5]	1.20 (1.06-1.36)	0.004	0	2.47	0.650
Garduño-Garcia Jde *et al*[6]	1.25 (1.09-1.44)	0.002	0	0.84	0.934
Lai *et al*[7]	1.21 (1.06-1.38)	0.005	0	2.34	0.673
Lai *et al*[8]	1.18 (1.04-1.35)	0.011	0	1.97	0.742
Liu *et al*[9]	1.15 (0.99-1.35)	0.071	0	1.97	0.742
Waring *et al*[10]	1.20 (1.03-1.36)	0.018	0	2.40	0.663

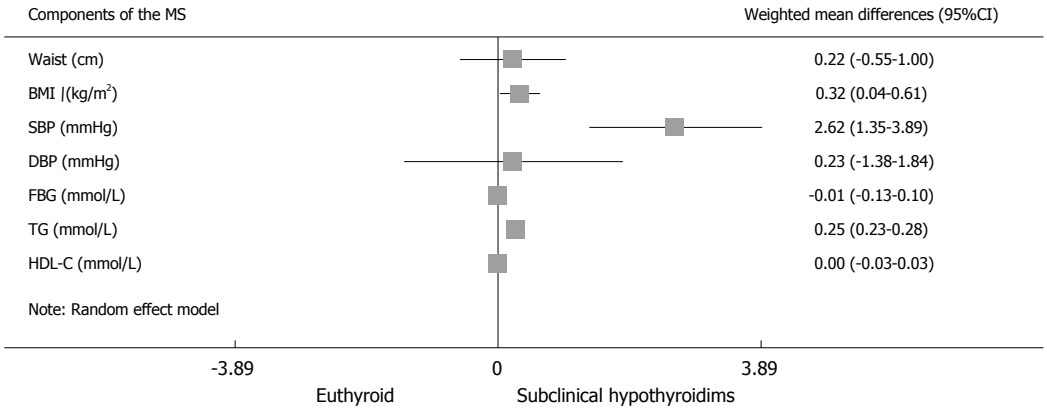

Figure 4 Weighted mean differences for the components of the metabolic syndrome. MS: Metabolic syndrome; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol.

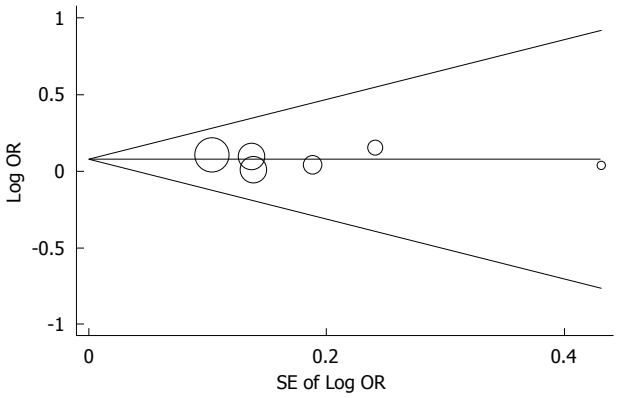

Figure 5 Begg's funnel plot with pseudo 95%CI for publication bias.

ence, DBP, fasting blood glucose and HDL-C, were not significantly different between the two groups (Figure 4).

The subgroup analysis indicated that the OR for the MS in Chinese studies did not significantly differ from that in non Chinese studies (OR for MS in Chinese studies: 1.256; 95%CI: 1.063-1.484; OR for MS in non Chinese studies: 1.130; 95%CI: 0.938-1.360, *P* for interaction = 0.404). The sensitivity analysis indicated that the pooled ORs excluding each individual study were comparable (Table 3). No significant publication bias was found after assessment using the Begg's funnel plot (*P* = 0.573) and the Egger weighted regression statistic (*P* = 0.833) (Figure 5).

DISCUSSION

In this meta-analysis of 19546 participants from six cross-sectional studies of a general population, SCH was associated with an increased risk of the MS compared with being euthyroid. To our best knowledge, this is the first meta-analysis showing the relationship between SCH and the MS. The previous meta-analysis demonstrated a possible association between SCH and increase risk of CHD[2]. This association might be mediated by the effect of these risk factors because the MS is one of the established risk factors for cardiovascular disease[4,16]. However, whether this association between SCH and coronary heart is independent or dependent of MS is still unclear and further investigation is needed.

Even in euthyroid populations, the relationship between thyroid function and the MS has been well investigated, with results consistent with those of our meta-analysis. In a cross-sectional study in the Netherlands, free T4 levels within the normal reference range were significantly related to four of the five components of the MS (*P* for waist circumference = 0.038, *P* for TGs = 0.023, *P* for HDL-C = 0.007 and *P* for SBP = 0.019), independent of insulin resistance[17]. Park *et al*[18] found a close relationship between TSH and the MS in euthyroid postmenopausal women (adjusted OR for the MS 1.55; 95%CI: 1.26-1.89; *P* < 0.001).

It was previously reported that overt hypothyroidism was associated with the MS[19], presumably because of the associated hypercholesterolemia and hypertension[20]. The reason why SCH is associated with the MS could be also attributed to the increased risk of the components of the MS. In a study evaluating 27097 individuals > 40 years of age without a diagnosis of thyroid disease, serum TSH, even within the normal range, was positively associated

with BMI (*P* for trend in all BMI groups < 0.001)[21]. The relationship between blood pressure and thyroid function has also been well investigated but with conflicting findings[22-25]. A recent meta-analysis of seven cross-sectional studies concluded that SCH is associated with increased blood pressure, whereas subclinical hyperthyroidism is not[26]. Hypothyroidism has been associated with dyslipidemia, which is characterized by increased levels of total cholesterol and low-density lipoprotein cholesterol[27]. In the Colorado thyroid disease prevalence study, the total cholesterol, low-density lipoprotein cholesterol and TG levels of SCH subjects were significantly greater than the corresponding lipid levels in euthyroid subjects ($P < 0.001$ for total cholesterol and low-density lipoprotein cholesterol; $P = 0.02$ for TG), whereas no difference was found in HDL-C level[28]. Our meta-analysis of metabolic components has further confirmed these conclusions.

Another important issue is whether SCH should be treated with thyroid hormones or whether hormone replacement therapy could reduce the cardiovascular risk and mortality in SCH. A systematic review of randomized controlled trials showed that thyroid hormone replacement in SCH could improve the lipid profile (WMD for low-density lipoprotein cholesterol: -10.77 mg/dL; 95%CI: -21.57-0.04; $P = 0.051$)[29] and recent data from a cohort study indicated that treatment of SCH with levothyroxine was associated with less ischemic heart disease in younger individuals compared to those without treatment (adjusted hazard ratio 0.61; 95%CI: 0.39-0.95)[30]. Since our meta-analysis indicated a higher risk of the MS in SCH, screening and treatment of these metabolic components are necessary to further reduce the risk of cardiovascular events.

It is notable that the included study from Waring *et al*[10] reported not only cross-sectional data but also prospective data, making it the only prospective study on SCH and the MS in the literature. After 6 years of follow-up, the risk of the MS in SCH individuals with a TSH > 10 mIU/L was similar to that in the baseline cross-sectional analysis but with a wider confidence interval (OR for cross-sectional analysis, 2.3; 95%CI: 1.0-5.0; OR for prospective analysis, 2.2; 95%CI: 0.6-7.5). However, this study seemed to be underpowered because a very small number of participants with marked SCH were included[10].

Our study has several limitations. Firstly, although our heterogeneity test showed no statistically significant heterogeneity among studies, it is still impossible that our final results would be confounded by the different definitions of SCH and MS among the included studies, as well as the different inclusion/exclusion criteria. Secondly, this is a study-level and we used unadjusted OR instead of adjusted OR; thus, it is impossible to exclude the confounding effects of age, sex and race. Finally, although no statistically significant publication bias was detected in our analysis, we believe there may be data relevant to this topic that have never been published.

Our meta-analysis indicated that SCH may be associated with an increased risk of the MS, which is attributed to the increased risk of metabolic components. A large prospective cohort study is needed to further confirm the conclusion of our study.

COMMENTS

Background

Over the past several decades, a plethora of publications has established that subclinical hypothyroidism (SCH) is associated with cardiovascular disease. Meanwhile, it has been well recognized that the metabolic syndrome (MS) is associated with increased cardiovascular events and all-cause mortality.

Research frontiers

Is SCH associated with a higher risk of the MS?

Innovations and breakthroughs

This meta-analysis of 6 cross-sectional studies with 19546 participants indicated that SCH may be associated with increased risk of the MS, which is attributed to the increased risk of metabolic components.

Applications

It may be necessary to screen for the MS in SCH individuals in clinical practice.

Terminology

SCH occurs when thyroid-stimulating hormone levels are elevated but thyroxine (T4) and triiodothyronine (T3) levels are normal. MS is a name for a group of risk factors that occur together and increase the risk for coronary artery disease, stroke and type 2 diabetes.

Peer review

This paper reports the results of a meta-analysis of observational studies evaluating the association between SCH and MS and its individual components. Overall methodology is correct and the results are very interesting and valuable.

REFERENCES

1 **Cooper DS**, Biondi B. Subclinical thyroid disease. *Lancet* 2012; **379**: 1142-1154 [PMID: 22273398 DOI: 10.1016/S0140-6736(11)60276-6]

2 **Rodondi N**, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Collet TH, Bremner A, Maisonneuve P, Sgarbi JA, Khaw KT, Vanderpump MP, Newman AB, Cornuz J, Franklyn JA, Westendorp RG, Vittinghoff E, Gussekloo J. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. *JAMA* 2010; **304**: 1365-1374 [PMID: 20858880 DOI: 10.1001/jama.2010.1361]

3 **Eckel RH**, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2010; **375**: 181-183 [PMID: 20109902 DOI: 10.1016/S0140-6736(09)61794-3]

4 **Mottillo S**, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; **56**: 1113-1132 [PMID: 20863953 DOI: 10.1016/j.jacc.2010.05.034]

5 **Hergenç G**, Onat A, Albayrak S, Karabulut A, Türkmen S, Sari I, Can G. TSH levels in Turkish adults: Prevalences and associations with serum lipids, coronary heart disease and metabolic syndrome. *Turk J Med Sci* 2005; **35**: 297-304

6 **Garduño-Garcia Jde J**, Alvirde-Garcia U, López-Carrasco G, Padilla Mendoza ME, Mehta R, Arellano-Campos O, Choza R, Sauque L, Garay-Sevilla ME, Malacara JM, Gomez-Perez FJ, Aguilar-Salinas CA. TSH and free thyroxine concentrations are associated with differing metabolic markers in euthyroid subjects. *Eur J Endocrinol* 2010; **163**: 273-278 [PMID: 20516204 DOI: 10.1530/EJE-10-0312]

7 **Lai CC**, Tang SH, Pei D, Wang CY, Chen YL, Wu CZ, Hsiao FC, Chen HS, Wang JY. The prevalence of subclinical thyroid dysfunction and its association with metabolic syndrome in Taiwanese elderly. *Int J Gerontol* 2011; **5**: 25-29

8 **Lai Y**, Wang J, Jiang F, Wang B, Chen Y, Li M, Liu H, Li C, Xue H, Li N, Yu J, Shi L, Bai X, Hou X, Zhu L, Lu L, Wang S,

Xing Q, Teng X, Teng W, Shan Z. The relationship between serum thyrotropin and components of metabolic syndrome. *Endocr J* 2011; **58**: 23-30 [PMID: 21135510 DOI: 10.1507/endocrj.K10E-272]

9 **Liu C**, Scherbaum WA, Schott M, Schinner S. Subclinical hypothyroidism and the prevalence of the metabolic syndrome. *Horm Metab Res* 2011; **43**: 417-421 [PMID: 21512964 DOI: 10.1055/s-0031-1275719]

10 **Waring AC**, Rodondi N, Harrison S, Kanaya AM, Simonsick EM, Miljkovic I, Satterfield S, Newman AB, Bauer DC. Thyroid function and prevalent and incident metabolic syndrome in older adults: the Health, Ageing and Body Composition Study. *Clin Endocrinol* (Oxf) 2012; **76**: 911-918 [PMID: 22187968 DOI: 10.1111/j.1365-2265.2011.04328.x]

11 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670 DOI: 10.1001/jama.283.15.2008]

12 **Liberati A**, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Ann Intern Med* 2009; **151**: W65-W94 [PMID: 19622512 DOI: 10.7326/0003-4819-151-4-200908180-00136]

13 **Rostom A**, Dubé C, Cranney A. Celiac Disease. Rockville, MD: Agency for Healthcare Research and Quality (US), 2004. (Evidence Reports/Technology Assessments, No. 104.) Appendix D. Quality Assessment Forms. Available from: URL: http://www.ncbi.nlm.nih.gov/books/NBK35156/

14 **Begg CB**, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**: 1088-1101 [PMID: 7786990 DOI: 10.2307/2533446]

15 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]

16 **Ford ES**. Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. *Diabetes Care* 2005; **28**: 1769-1778 [PMID: 15983333 DOI: 10.2337/diacare.28.7.1769]

17 **Roos A**, Bakker SJ, Links TP, Gans RO, Wolffenbuttel BH. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *J Clin Endocrinol Metab* 2007; **92**: 491-496 [PMID: 17090642 DOI: 10.1210/jc.2006-1718]

18 **Park HT**, Cho GJ, Ahn KH, Shin JH, Hong SC, Kim T, Hur JY, Kim YT, Lee KW, Kim SH. Thyroid stimulating hormone is associated with metabolic syndrome in euthyroid postmenopausal women. *Maturitas* 2009; **62**: 301-305 [PMID: 19250778 DOI: 10.1016/j.maturitas.2009.01.007]

19 **Erdogan M**, Canataroglu A, Ganidagli S, Kulaksızoglu M. Metabolic syndrome prevalence in subclinic and overt hypothyroid patients and the relation among metabolic syndrome parameters. *J Endocrinol Invest* 2011; **34**: 488-492 [PMID: 20651468]

20 **Klein I**, Ojamaa K. Thyroid hormone and the cardiovascular system. *N Engl J Med* 2001; **344**: 501-509 [PMID: 11172193 DOI: 10.1056/NEJM200102153440707]

21 **Asvold BO**, Bjøro T, Vatten LJ. Association of serum TSH with high body mass differs between smokers and never-smokers. *J Clin Endocrinol Metab* 2009; **94**: 5023-5027 [PMID: 19846737 DOI: 10.1210/jc.2009-1180]

22 **Iqbal A**, Figenschau Y, Jorde R. Blood pressure in relation to serum thyrotropin: The Tromsø study. *J Hum Hypertens* 2006; **20**: 932-936 [PMID: 17024137 DOI: 10.1038/sj.jhh.1002091]

23 **Duan Y**, Peng W, Wang X, Tang W, Liu X, Xu S, Mao X, Feng S, Feng Y, Qin Y, Xu K, Liu C, Liu C. Community-based study of the association of subclinical thyroid dysfunction with blood pressure. *Endocrine* 2009; **35**: 136-142 [PMID: 19130316 DOI: 10.1007/s12020-008-9138-y]

24 **Chen H**, Xi Q, Zhang H, Song B, Liu X, Mao X, Li J, Shen H, Tang W, Zhang J, Wang Z, Duan Y, Liu C. Investigation of thyroid function and blood pressure in school-aged subjects without overt thyroid disease. *Endocrine* 2012; **41**: 122-129 [PMID: 21986920 DOI: 10.1007/s12020-011-9517-7]

25 **Walsh JP**, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, Feddema P, Michelangeli V. Subclinical thyroid dysfunction and blood pressure: a community-based study. *Clin Endocrinol* (Oxf) 2006; **65**: 486-491 [PMID: 16984241 DOI: 10.1111/j.1365-2265.2006.02619.x]

26 **Cai Y**, Ren Y, Shi J. Blood pressure levels in patients with subclinical thyroid dysfunction: a meta-analysis of cross-sectional data. *Hypertens Res* 2011; **34**: 1098-1105 [PMID: 21796125 DOI: 10.1038/hr.2011.91]

27 **Duntas LH**. Thyroid disease and lipids. *Thyroid* 2002; **12**: 287-293 [PMID: 12034052 DOI: 10.1089/10507250252949405]

28 **Canaris GJ**, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. *Arch Intern Med* 2000; **160**: 526-534 [PMID: 10695693 DOI: 10.1001/archinte.160.4.526]

29 **Villar HC**, Saconato H, Valente O, Atallah AN. Thyroid hormone replacement for subclinical hypothyroidism. *Cochrane Database Syst Rev* 2007; CD003419 [PMID: 17636722]

30 **Razvi S**, Weaver JU, Butler TJ, Pearce SH. Levothyroxine treatment of subclinical hypothyroidism, fatal and nonfatal cardiovascular events, and mortality. *Arch Intern Med* 2012; **172**: 811-817 [PMID: 22529180 DOI: 10.1001/archinternmed.2012.1159]

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MINIREVIEWS

Duplicates in systematic reviews: A critical, but often neglected issue

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Abstract

The number of systematic reviews is gradually increasing over time. Also, the methods to perform a systematic review are being improved. However, little attention has been paid for the issue regarding how to find duplicates in systematic reviews. On the basis of the survey and systematic reviews by our team and others, we review the prevalence, significance and classification of duplicates and the method to find duplicates in a systematic review. Notably, although a preliminary method to find duplicates is established, its usefulness and convenience need to be further confirmed.

Key words: Duplicates; Systematic review; Method; Prevalence; Significance

Core tip: Finding duplicates is an indispensable step in a systematic review. The prevalence of duplicates ranges from 7% to 25%, as three different databases were searched (PubMed, EMBASE, Cochrane library). Until now, few studies have reported the detailed information regarding how to find duplicates. A preliminary method to find duplicates is established, but its usefulness and convenience need to be further confirmed.

Qi XS, Bai M, Yang ZP, Ren WR. Duplicates in systematic reviews: A critical, but often neglected issue. *World J Meta-Anal* 2013; 1(3): 97-101 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i3/97.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i3.97

INTRODUCTION

Nowadays, there is an increasing trend in the number of systematic reviews over time (Figure 1). This phenomenon is primarily because well-conducted systematic reviews can provide the best-quality research evidence for clinicians by combining the results of all available data. The PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement, an updated version of Quality of Reporting of Meta-analysis statement published in 1999[1], has been recently developed to improve the quality of reporting systematic reviews[2]. More recently, an international registry of systematic review protocols is being established by the Centre for Reviews and Dissemination to further increase the transparency of the review process and outcomes[3]. In spite of these methodological advances, little attention has been paid for the issue regarding how to find duplicates in systematic reviews. On the basis of the survey and systematic reviews by our team and others, this review primarily aims to outline the prevalence, significance, and classification of duplicates, and to describe the method regarding how to find duplicates in a systematic review.

PREVALENCE

In this section, the prevalence of duplicates reported in

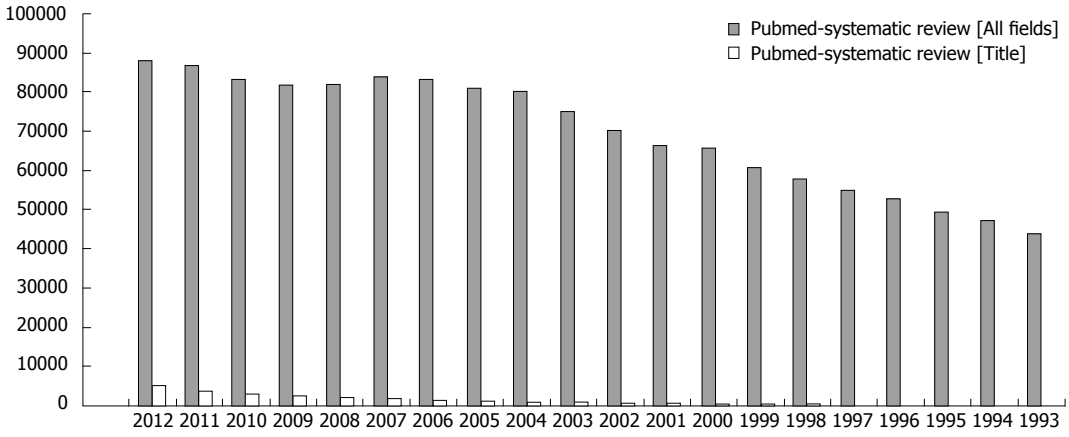

Figure 1 Trend in the publication of systematic reviews over time.

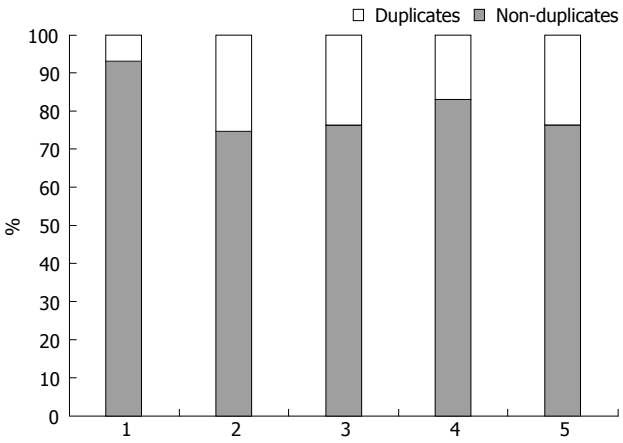

Figure 2 The prevalence of duplicates reported in four systematic reviews conducted by our team. 1: Qi JGH (2013); 2: Bai JGH (2013); 3: Qi AJM (2013); 4: Qi PlosOne (2013) PVT; 5: Qi PlosOne (2013) BCS.

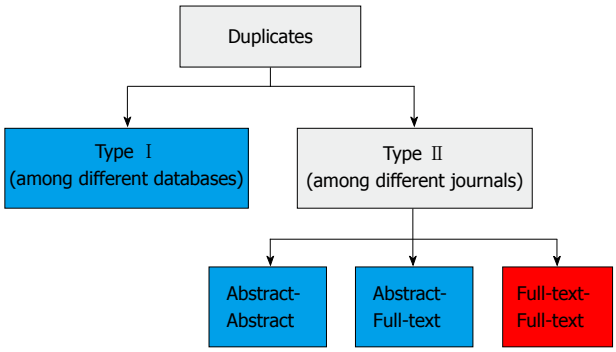

Figure 3 Classification of duplicates proposed by our team. Types of duplicates signed by blue are often permitted, but types of duplicates signed by red are unethical.

four systematic reviews conducted by our team using more than three databases is summarized[4-7] (Figure 2). The reason why only the literature search results from our published papers are primarily reviewed is primarily because of the reliability and accuracy of data regarding the duplicates in our systematic reviews. Additionally, other systematic reviews using only one database are not analyzed[8-11]. Overall, the prevalence of duplicates ranges from 7% to 25% among the three different databases (PubMed, EMBASE, Cochrane library).

On the other hand, several investigators have evaluated the duplicate publication in different journals or specialties. Arrivé *et al*[12] searched 362 original research articles published in Radiology during 2001, but only two redundant publications were found among these articles. Durani also found that only less than 1% of articles had some degree of redundancy in British Journal of Plastic Surgery and Plastic and Reconstructive Surgery during 2000[13]. By comparison, Schein and Paladugu screened 660 original articles published in the journals Surgery, The British Journal of Surgery and Archives of Surgery during 1998 and found that about 14% of these papers had some potential form of a redundant publication[14].

Recently, our systematic analysis demonstrated a relatively high prevalence of covert duplicate publications among articles on Budd-Chiari syndrome in China (10%, 184/1914)[6]. Notably, we also found a significantly higher prevalence of covert duplicate publications in Science Citation Index journals, compared to Chinese language journals.

SIGNIFICANCE

The most detrimental effect of duplicates on the systematic review is to introduce the potential bias and to influence the reliability of the conclusion. In a case study by Tramèr *et al*[15], all published randomized controlled trials, which compared the prophylactic and therapeutic efficacy between ondansetron and placebo, no treatment, or other antiemetics on nausea and vomiting after general anesthesia, were retrieved by searching Medline, EMBASE, and Biological Abstracts databases. The investigators found a high prevalence of duplicate papers (17% of randomized controlled trials and 28% of patient data were duplicated). More importantly, if duplicated data were included into the meta-analysis, the antiemetic efficacy of ondansetron would be overestimated by 23%. In addition, covert duplicate publications can cause some serious harm in routine clinical practice[16,17], such as vio-

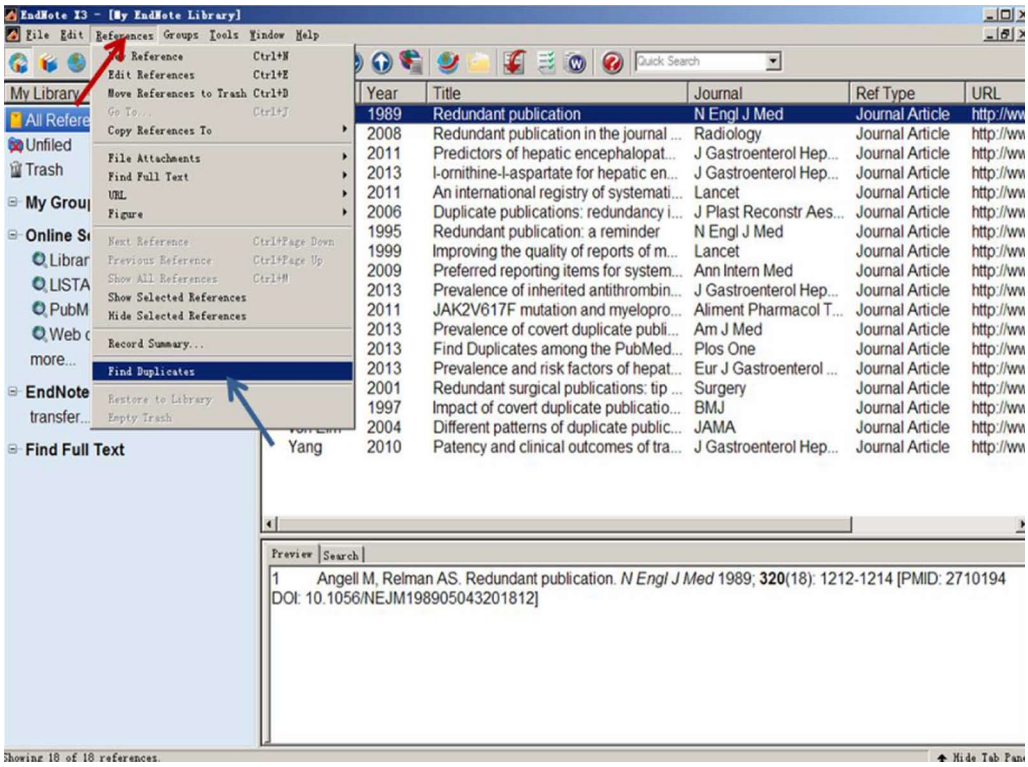

Figure 4 Use of "Find Duplicates" command under the "References" menu. Red arrow indicates the "References" menu. Blue arrow indicates the "Find Duplicates" command.

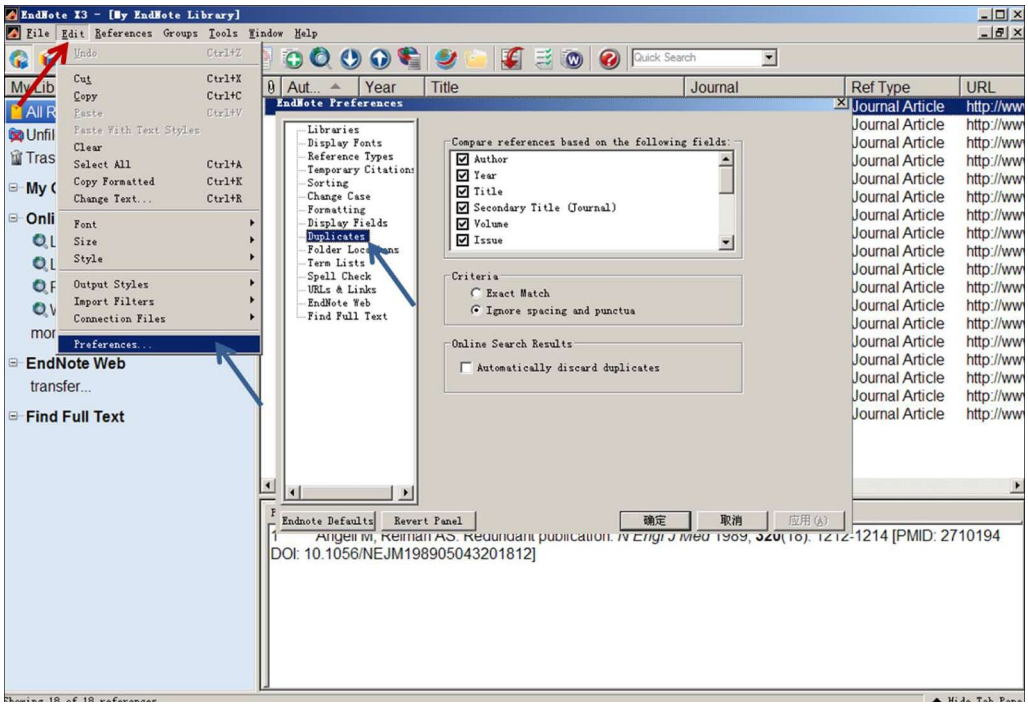

Figure 5 Use of the "Preferences" command under the "Edit" menu. Red arrow indicates the "Edit" menu. Blue arrow indicates the "Preferences" and "Duplicates" commands.

lation of the ethics of scientific publications and inflation of medical knowledge.

CLASSIFICATION

Several different classifications or patterns of duplicates have been proposed.

First, a traditional grading system includes three types of redundant publications, as follows: (1) dual publication (identical material, methods, and conclusions); (2) potentially dual (almost identical material, methods, and conclusions); and (3) salami slicing (suspected study rep-

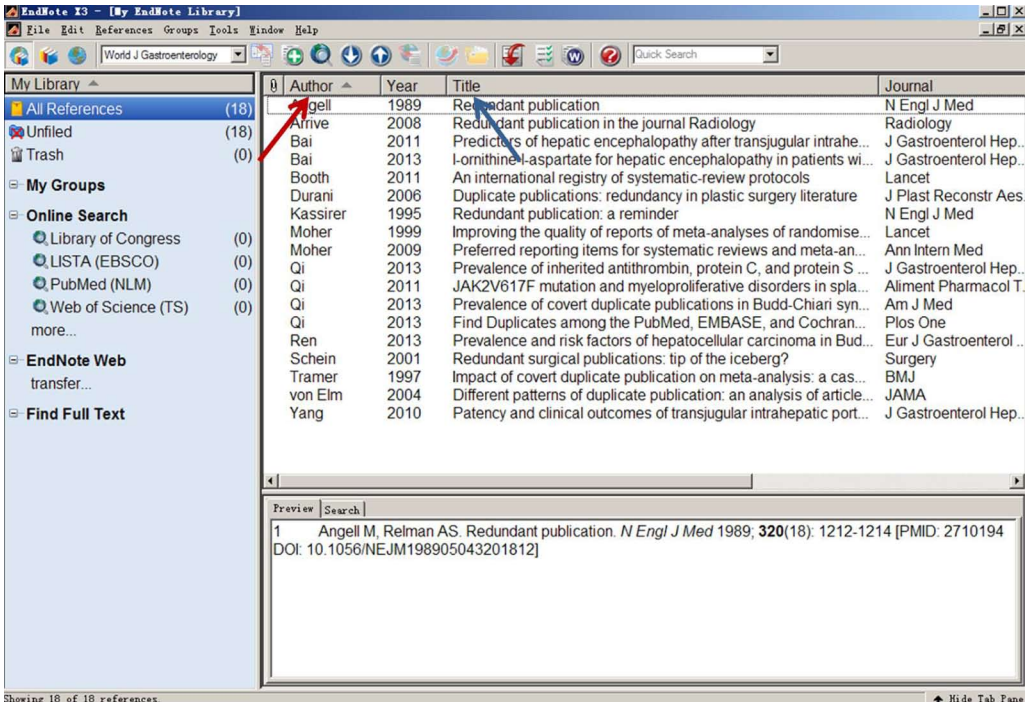

Figure 6 Ordering the literatures according to the first author's name and title. Red arrow indicates that literatures are ordered according to the author's name. Blue arrow indicates that literatures are ordered according to the title.

resents a part of, continuation of, or partial repetition of the index article)[14]. This grading system has been widely used by journal editors and peer reviewers to identify the redundant publications[14].

Second, a Switzerland group has recently identified six different patterns of duplicate publications by analyzing 56 systematic reviews in anesthesia and analgesia[18]. According to the samples and outcomes, they include: (1) identical samples and identical outcomes; (2) same as 1 but several duplicates assembled; (3) identical samples and different outcomes; (4) increasing samples and identical outcomes; (5) decreasing samples and identical outcomes; and (6) different samples and different outcomes. Systematic review authors should be encouraged to use this classification to unearth duplications and make them public.

Finally, more recently, our group has further divided the duplicates into two main types by analyzing the literature regarding portal vein thrombosis and Budd-Chiari syndrome in systematic reviews[7] (Figure 3). Type Ⅰ duplicates would be considered, if one paper was simultaneously recorded in one database twice or more times or in two or three databases[7]. Type Ⅱ duplicates would be considered, if one study was published in different journals or issues[7]. The simple classification is primarily dependent on the origin of redundant publications (different databases or different journals). Certainly, the type Ⅰ duplicates are reasonable. In addition, according to the type of publication, type Ⅱ duplicates are further classified as Abstract-Abstract, Abstract-Full text and Full text-Full text[7]. The first two types are often permitted, but the last type is unethical and considered as redundant publication.

HOW TO FIND DUPLICATES?

Although it is a critical issue to find duplicates, no consensus regarding how to find duplicates has been reached yet. In an analysis of previous systematic reviews, the investigators described a decision tree to mutually identify the patterns of duplicate publications, but did not introduce any methods to find duplicates[18]. Our group attempted to design a pragmatic strategy of combining auto- and hand-searching duplicates in systematic reviews. Briefly, this strategy includes two main steps as follows[7]. First, the review authors can use "Find Duplicates" command on the "References" menu to automatically identify the duplicates in the Endnote library (Figure 4). Notably, before auto-searching duplicates, the "Preferences" command on the "Edit" menu could be used to edit the characteristics of duplicates (Figure 5). Additionally, all identified duplicates should be confirmed by review authors. Second, the remaining literatures are ordered according to the first author's name and title (Figure 6). Review authors can mutually identify the duplicates by the same author and title. Certainly, further confirmation of accuracy of these duplicates should be necessary. Additionally, we have to acknowledge that not all duplicates would be found by the above-mentioned method, especially when the first authors and titles are different between two potential duplicate papers. Thus, further improvement of this method to find duplicates should be warranted.

CONCLUSION

As we have known, literature search results are often variable across different databases, thereby potentially leading to heterogeneous conclusions. Therefore, it has been recommended that multiple databases should be adopted to search the relevant literatures in a systematic review[19]. In this case, further work is very necessary to find duplicates among different databases. However, few systematic reviews reported the detailed information regarding how to find duplicates. A preliminary method to find duplicates is established, but its usefulness and convenience need to be further confirmed.

REFERENCES

1 **Moher D**, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. Quality of Reporting of Meta-analyses. *Lancet* 1999; **354**: 1896-1900 [PMID: 10584742]

2 **Moher D**, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009; **151**: 264-269, W64 [PMID: 19622511]

3 **Booth A**, Clarke M, Ghersi D, Moher D, Petticrew M, Stewart L. An international registry of systematic-review protocols. *Lancet* 2011; **377**: 108-109 [PMID: 20630580]

4 **Qi X**, De Stefano V, Wang J, Bai M, Yang Z, Han G, Fan D. Prevalence of inherited antithrombin, protein C, and protein S deficiencies in portal vein system thrombosis and Budd-Chiari syndrome: a systematic review and meta-analysis of observational studies. *J Gastroenterol Hepatol* 2013; **28**: 432-442 [PMID: 23216127 DOI: 10.1111/jgh.12085]

5 **Bai M**, Yang Z, Qi X, Fan D, Han G. l-ornithine-l-aspartate for hepatic encephalopathy in patients with cirrhosis: a meta-analysis of randomized controlled trials. *J Gastroenterol Hepatol* 2013; **28**: 783-792 [PMID: 23425108 DOI: 10.1111/jgh.12142]

6 **Qi X**, Ren W, Liu L, Yang Z, Yang M, Fan D, Han G. Prevalence of covert duplicate publications in Budd-Chiari syndrome articles in China: a systematic analysis. *Am J Med* 2013; **126**: 633-639.e2 [PMID: 23787196]

7 **Qi X**, Yang M, Ren W, Jia J, Wang J, Han G, Fan D. Find duplicates among the PubMed, EMBASE, and Cochrane Library Databases in systematic review. *PLoS One* 2013; **8**: e71838 [PMID: 23977157 DOI: 10.1371/journal.pone.0071838]

8 **Qi X,** Yang Z, Bai M, Shi X, Han G, Fan D. Meta-analysis: the significance of screening for JAK2V617F mutation in Budd-Chiari syndrome and portal venous system thrombosis. *Aliment Pharmacol Ther* 2011; **33**: 1087-1103 [PMID: 21395632 DOI: 10.1111/j.1365-2036.2011.04627.x]

9 **Ren W**, Qi X, Yang Z, Han G, Fan D. Prevalence and risk factors of hepatocellular carcinoma in Budd-Chiari syndrome: a systematic review. *Eur J Gastroenterol Hepatol* 2013; **25**: 830-841 [PMID: 23411869 DOI: 10.1097/MEG.0b013e32835eb8d4]

10 **Bai M**, Qi X, Yang Z, Yin Z, Nie Y, Yuan S, Wu K, Han G, Fan D. Predictors of hepatic encephalopathy after transjugular intrahepatic portosystemic shunt in cirrhotic patients: a systematic review. *J Gastroenterol Hepatol* 2011; **26**: 943-951 [PMID: 21251067 DOI: 10.1111/j.1440-1746.2011.06663.x]

11 **Yang Z**, Han G, Wu Q, Ye X, Jin Z, Yin Z, Qi X, Bai M, Wu K, Fan D. Patency and clinical outcomes of transjugular intrahepatic portosystemic shunt with polytetrafluoroethylene-covered stents versus bare stents: a meta-analysis. *J Gastroenterol Hepatol* 2010; **25**: 1718-1725 [PMID: 21039832 DOI: 10.1111/j.1440-1746.2010.06400.x]

12 **Arrivé L**, Lewin M, Dono P, Monnier-Cholley L, Hoeffel C, Tubiana JM. Redundant publication in the journal Radiology. *Radiology* 2008; **247**: 836-840 [PMID: 18403625 DOI: 10.1148/radiol.2473070969]

13 **Durani P**. Duplicate publications: redundancy in plastic surgery literature. *J Plast Reconstr Aesthet Surg* 2006; **59**: 975-977 [PMID: 16920591]

14 **Schein M**, Paladugu R. Redundant surgical publications: tip of the iceberg? *Surgery* 2001; **129**: 655-661 [PMID: 11391360]

15 **Tramèr MR**, Reynolds DJ, Moore RA, McQuay HJ. Impact of covert duplicate publication on meta-analysis: a case study. *BMJ* 1997; **315**: 635-640 [PMID: 9310564]

16 **Kassirer JP**, Angell M. Redundant publication: a reminder. *N Engl J Med* 1995; **333**: 449-450 [PMID: 7616995 DOI: 10.1056/NEJM199508173330709]

17 **Angell M**, Relman AS. Redundant publication. *N Engl J Med* 1989; **320**: 1212-1214 [PMID: 2710194 DOI: 10.1056/NEJM198905043201812]

18 **von Elm E**, Poglia G, Walder B, Tramèr MR. Different patterns of duplicate publication: an analysis of articles used in systematic reviews. *JAMA* 2004; **291**: 974-980 [PMID: 14982913 DOI: 10.1001/jama.291.8.97410.1001/jama.291.8.974]

19 **Wu YP**, Aylward BS, Roberts MC, Evans SC. Searching the scientific literature: implications for quantitative and qualitative reviews. *Clin Psychol Rev* 2012; **32**: 553-557 [PMID: 22819996]

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META-ANALYSIS

Preventing pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections with probiotics: A meta-analysis

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Abstract

AIM: To assess the efficacy and safety of probiotics for preventing pediatric: (1) antibiotic associated diarrhea and (2) *Clostridium difficile* (*C. difficile*) infections.

METHODS: On June 3, 2013, we searched PubMed (1960-2013), EMBASE (1974-2013), Cochrane Database of Systematic Reviews (1990-2013), CINAHL (1981-2013), AMED (1985-2013), and ISI Web of Science (2000-2013). Additionally, we conducted an extensive grey literature search including contact with National Institutes of Health Clinical Trials Registry, abstracts from annual infectious disease and gastroenterology meetings, experts in the field and correspondence with authors. The primary outcomes were the incidence of antibiotic-associated diarrhea (AAD) and *C. difficile* infections (CDI). Dichotomous outcomes (*e.g.*, incidence of AAD or CDI) were pooled using a random-effects model to calculate the relative risk and corresponding 95% confidence interval (95%CI) and weighted on study quality. To explore possible explanations for heterogeneity, a *priori* subgroup analysis were conducted on probiotic strain type, daily dose, quality of study and safety of probiotics. The overall quality of the evidence supporting each outcome was assessed using the grading of recommendations, assessment, development and evaluation criteria.

RESULTS: A total of 1329 studies were identified with 22 trials (23 treatment arms and 4155 participants) meeting eligibility requirements for our review of prevention of AAD and 5 trials (1211 participants) for the prevention of CDI. Trials in adult populations, trials of uncertain antibiotic exposure or studies which did not provide incidence of AAD were excluded. We found 12 trials testing a single strain of probiotic and 10 trials testing a mixture of probiotic strains. Probiotics (all strains combined) significantly reduced the incidence of pediatric AAD (pooled RR = 0.42, 95%CI: 0.33-0.53) and significantly reduced pediatric CDI (pooled RR = 0.35, 95%CI: 0.13-0.92). Of the two strains with multiple trials, both significantly reduced pediatric AAD: *Saccharomyces boulardii* lyo (pooled RR = 0.43, 95%CI: 0.32-0.60) and *Lactobacillus rhamnosus* GG (pooled RR = 0.36, 95%CI: 0.19-0.69). There was no significant effect by type of antibiotic, or by duration or dose of probiotic. No adverse events associated were found in the 22 controlled trials relating to the use of probiotics.

CONCLUSION: This meta-analysis found that probiotics significantly prevented pediatric antibiotic associated diarrhea and pediatric CDI, but the efficacy varies significantly by the strain of the probiotic.

Key words: Probiotics; Pediatric; Antibiotic-associated diarrhea; *Clostridium difficile*; *Saccharomyces boulardii*; *Lactobacillus rhamnosus*; Safety; Meta-analysis; Randomized clinical trials

Core tip: A meta-analysis was conducted (1985-2013) for clinical trials testing probiotics for the prevention of pediatric antibiotic-associated diarrhea (AAD) or *Clostridium difficile* infections (CDI). Overall, probiotics significantly reduced the incidence of pediatric AAD (pooled from 22 trials RR = 0.42, 95%CI: 0.33-0.53) and significantly reduced pediatric CDI (pooled from five trials RR = 0.35, 95%CI: 0.13-0.92). Of the two strains with multiple trials, both significantly reduced pediatric AAD: *Saccharomyces boulardii* lyo (RR = 0.43, 95%CI: 0.32-0.60) and *Lactobacillus rhamnosus* GG (RR = 0.36, 95%CI: 0.19-0.69). There was no significant effect by type of antibiotic, or by duration or dose of probiotic.

McFarland LV, Goh S. Preventing pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections with probiotics: A meta-analysis. *World J Meta-Anal* 2013; 1(3): 102-120 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i3/102.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i3.102

INTRODUCTION

The use of antibiotics, while effective in treating a precipitating infection, may cause diarrheal disease as a common side effect, termed antibiotic associated diarrhea (AAD), caused by the unintended disruption of normal intestinal flora. Normal microbiota is a complex interaction of bacterial and fungal species that produce a phenomenum called "colonization resistance", which acts as a barrier to opportunistic pathogens. The most commonly known etiology of AAD is *Clostridium difficile* (*C. difficile*), which takes advantage of the disruption of colonization resistance and overgrows the intestines, producing toxins and resulting in an inflammatory intestinal disease called *C. difficile* infection (CDI)[1]. These unintended consequences of antibiotic use are well-studied phenomena in adults, but less attention has been focused on the pediatric population. Pediatric patients present unique challenges for the clinical management of disease due to differences in their immune development, susceptibility to dehydration and their response to treatments.

Pediatric AAD and pediatric CDI were recognized as important clinical concerns as the incidence of both continues to increase over time and serious consequences of infection are reported[2]. The incidence of pediatric AAD varies widely from 6%-11% in pediatric outpatients to 23%-33% in pediatric inpatients[1,3]. Data collected from national United States surveys of pediatric inpatients shows the incidence of pediatric CDI has increased 2.5 fold over three years, from 12.8/10000 in 2006[4] to 31.5/10000 in 2009[5]. A more recent study in 41 children's hospitals found pediatric CDI at 73/10000[6].

Clinical symptoms include asymptomatic carriage of *C. difficile* (typically 65% of neonates carry *C. difficile* but do not develop symptoms), mild-moderate diarrhea is most common in infants and older children (typically peaking at age 2-6 years old), to more severe disease (colitis or pseudomembraneous colitis) less frequently and rarely toxic megacolon[7,8]. As with adults, nearly 20% of children with one episode may develop recurrent episodes of CDI[7]. Consequences of pediatric AAD or CDI may include a 2-3 fold increase in length of hospital stay[5,6], a 6-fold increase in the risk of mortality[5,6], and the need for colectomy (approximately 2%)[8,9].

Current recommended treatments for pediatric AAD and CDI include discontinuation of the inciting antibiotic if possible (for mild diarrhea) or treatment with metronidazole or vancomycin, however treatment failure is common (18% with metronidazole) and vancomycin is used with caution in children due to toxicity[7,10]. Cases of moderate-severe pediatric diarrhea often require the administration of oral rehydration therapy or parental fluids to reduce dehydration associated with diarrhea. Alternative strategies are currently being sought to prevent pediatric AAD and CDI, rather than delaying until the children are ill.

Probiotics are living microorganisms, which when administered in adequate amounts, confer a health benefit to the host[11]. The use of probiotics may be especially suited for AAD and CDI, as they are linked by a common mechanism of action, namely interactions with the normal microflora[2]. When antibiotics disrupt colonization resistance, overgrowth of pathogens may occur and disease erupts. Probiotics act as surrogate normal flora to protect the intestine until the normal microbiota can recover (typically 1-2 mo, after antibiotics are discontinued)[1]. Some probiotics also have other mechanisms of action (production of bacteriocins, stimulation of the immune response, production of toxin-destroying proteases, attachment site interference, *etc.*) that are also beneficial to the pediatric patient[12]. While over 60 clinical trials testing probiotics for AAD and/or CDI have been reported, most (66%) have been done in the adult population, so the efficacy of probiotics for children is less well documented. Evidence from meta-analyses of AAD and CDI have indicated probiotics, in general, may be efficacious for the prevention of these diseases, but they have based their results on mixed adult-pediatric populations, or have been based on adults only[13-15]. Because the efficacy to prevent AAD and CDI has been determined to be specific by probiotic strain, it is imperative that data are analyzed by separate strains.

The purpose of this meta-analysis to evaluate the efficacy and safety of similar probiotic strains for the prevention of antibiotic associated diarrhea and *C. difficile* infections in the pediatric population.

MATERIALS AND METHODS

Study objectives

Primary aims: (1) to systematically assess whether probiotics co-administered with antibiotics (any agent) reduces the incidence of AAD in children; and (2) to systematically assess whether probiotics co-administered

with antibiotics (any type) reduces the incidence of CDI in children.

Secondary aims: (1) to assess the efficacy by specific strain of probiotic for the prevention of AAD and CDI in children; (2) to systematically assess if there is a dose effect for probiotics in the prevention of AAD and CDI in children; (3) to determine if study quality is associated with a change in the estimate of outcome effect; and (4) to assess the safety of the use of probiotics in children receiving antibiotics.

Criteria for study selection

Abstracts of all citations and retrieved studies were reviewed and rated for inclusion. Full articles were retrieved if probiotics were given to prevent AAD or CDI in a pediatric population, or if the population age range was unclear from the abstract. Inclusion criteria included randomized (well described or partially) controlled trials (either placebo, standard active treatments, or no treatment given), blinded or open trials in pediatric populations (inpatient or outpatients) published in peer-reviewed journals or on clinical trial websites. Non-English language trials were translated and included whenever possible. Exclusion criteria included pre-clinical studies, safety or phase 2 studies, adult patients or healthy volunteer populations, diarrhea not associated with antibiotic use, case reports or case series, duplicate reports, trials of unspecified types of probiotics, incomplete or no diarrheal outcomes reported, no data on incidence rates of AAD or CDI, mixed pediatric and adult patient populations or if translation could not be obtained.

Interventions

The type of probiotic intervention included probiotics in any form (*e.g.,* capsule, sachet, yogurt, wafer). Trials investigating non-specific probiotics or yogurts (*e.g.,* products that do not label the probiotic strain and dose) were excluded. Trials combining probiotics with prebiotics were included if the prebiotic dose was less than 2.5 g, as this was judged to be of limited impact to alter the intestinal microflora[16,17]. Trials not providing the dose of the prebiotic in the product were excluded. The type of control group may include: placebo, active treatment currently used as standard practice, or no treatment control.

Outcomes and definitions

The primary outcome for AAD is defined as diarrhea (typically definition varied from > 2-3 loose or watery stools/day for > 2 consecutive days) occurring within 2 mo of antibiotic use[14]. The primary outcome for CDI is defined as a new episode of diarrhea associated with a positive culture or toxin (A or B) assay within 1 mo of antibiotic use[14,18].

Data sources

On June 3, 2013, we searched PubMed (1960-2013), EMBASE (1974-2013), Cochrane Database of Systematic Reviews (1990-2013), CINAHL (1981-2013), AMED (1985-2013), and ISI Web of Science (2000-2013). Three on-line clinical trial registries were searched: Cochrane Central Register of Controlled trials (http://www.cochrane.org), MetaRegister of Controlled Trials (http:www.controlled-trials.com/mrct) and National Institutes of Health (http://www.clinicaltrials.gov). Additionally, we conducted an extensive grey literature search including abstracts from annual infectious disease and gastroenterology meetings, experts in the field and communication with published authors on pediatric AAD or CDI. Search terms included: Antibiotic-associated diarrhea, *C. difficile* disease and/or infection, pediatric, randomized controlled trial and probiotics and specific probiotic strains. Search strategies were broad-based initially, then narrowed to the disease and population of interest. The procedure of this meta-analysis follows MOOSE guidelines using clearly delineated parameters, a *priori* inclusion and exclusion criteria and standardized data extraction tools[19,20].

Data extraction

Two authors independently and in duplicate extracted data and assessed risk of bias using pre-constructed, and piloted, data extraction forms. Any disagreements were resolved by discussion. For articles published in abstract form only, further information was sought by contacting principal authors. Articles not published in the English language were translated. Using a standardized data extraction form, we systematically collected the following data: authors, year of publication and journal, pediatric population data (age range, setting, antibiotic given for disease, types of antibiotics given), study aims and outcomes, study methods (study design, eligibility criteria, sample size calculations, interim analysis, statistical methods used, recruitment methods, subgroup analysis done), randomization (method of randomization allocation, randomization method), degree of blinding (open, single or double), intervention data (probiotic strains used, daily dose, duration of treatment, duration of follow-up, type of control used, treatment concealment), results (balanced randomization achieved, attrition rate and reasons, comparison of treatment groups by demographics, *etc.*, CONSORT flow-chart provided), AAD outcome data (incidence of AAD by group, ITT or APP analysis used, method to assess AAD), CDI outcome data (incidence of CDI by group, ITT or APP analysis used, method to assess CDI), safety data (adverse events reported by group), discussion points (limitations, generalizability and comparison of study results to published papers), clinical trial registration, location of protocol, and source of funding.

Assessment of methodological quality

Quality components for each trial were assessed for selection, detection, performance, reporting and loss to follow-up bias. Each of the included studies was evaluated using 33 items collected with the standardized data extraction form. Each item was graded as: present, ab-

sent, or not applicable (for example studies done in countries not requiring clinical trial registration, CONSORT flow-chart not present if trial was published before this became a standard, *etc.*)[19]. The overall quality score for the trial was calculated as the percent of items present divided by the total items present and absent (not applicable items were excluded from the calculation). Each of the 33 quality items were analyzed within one of six categories of potential of bias: study design bias (trial title, setting, early stoppage, background, study aims, prospective design, eligibility criteria, sample size calculation, interim analysis, statistical methods, recruitment methods, subgroup methods, probiotic well described by strain, daily dose and duration), selection bias (randomization allocation method, balanced groups resulted), detection bias (double blinded, treatments concealment), attrition bias (rates provided and reasons by each group), reporting bias (baseline group comparison, CONSORT flow-chart, intent to treat analysis done for each AAD and CDI outcome, incidence of each outcome provided, adverse event data provided and sub-group analysis provided, if applicable) and miscellaneous sources of bias (limitations, generalizability and comparison with other studies in discussion, trial registration, location of protocol for access and source of funding, if appropriate). Trials were classified as high quality if > 75% of the quality items were present, moderate quality if 50%-75% were present and low quality of < 50% were present.

We also employed the GRADE (grading of recommendations, assessment, development and evaluation) system for rating overall quality of evidence for each of the outcomes (prevention of AAD or prevention of CDI) by probiotic strain or type (single strain compared to mixtures of strains)[21,22]. Recommendation for use of each probiotic strain or mixture can be assessed by the overall strength of the evidence ("strong", many randomized controlled trials show significant protection, more benefit than risk, cost-effective or "weak", only case series or reports, limited number of small trials, *etc.*). Quality of the evidence is graded as "high quality" (further research is unlikely to change our confidence in the estimate of the effect), or "moderate quality" (further research is likely to have an important impact on our confidence and may change the estimate of the effect), or "low quality" (further research is very likely to change our confidence in the estimate and may change the direction of the estimate of the effect).

Statistical analysis

Statistical analysis was performed using Stata software version 12 (Stata Corporation, College Station, Texas). The primary outcomes were the incidence of AAD and CDI. Univariate analysis of bivariate parameters were analyzed using χ^2 test or Fisher's exact test for small cell sizes (< 5) with a significance level of $P \leqslant 0.05$. Meta-analysis was conducted for dichotomous outcomes (*e.g.*, incidence of AAD or CDI) using models to calculate the pooled relative risk and corresponding 95% confidence interval (95%CI) and weighted by study quality score. Heterogeneity across trials was evaluated using Cochran Q test based on pooled relative risks by the Mantel-Haenszel method[23]. If the studies were homogenous, a fixed effects model was used, if studies were heterogeneous, a random effect model was employed. If significant heterogeneity was detected, a subgroup analysis was conducted to determine the source of heterogeneity. To explore possible explanations for heterogeneity, a *priori* subgroup analyses were conducted on study size, probiotic strain type, daily dose [$\geqslant 1 \times 10^{10}$ colony-forming units (cfu) per day or $< 1 \times 10^{10}$ cfu/d] and by quality of study.

Publication bias

To assess for publication bias, a funnel plot, as well as a weighted regression (Egger's test) and a rank correlation test (Begg's test for small study effects) were conducted[20,24]. If publication bias was apparent, adjustment of the pooled estimates was considered using the trim and fill method[25]. Funnel plots show graphically that as sample sizes of trials increase, the precision is estimating the underlying treatment effect increases, which results in the effect estimates (relative risks) from small trials scattering more widely at the bottom of the graph and narrower scattering among larger studies. In the absence of publication bias, the funnel plot resembles a symmetrical inverted funnel. Reporting bias (smaller studies showing no protective effect) often are not published, and are indicated by an asymmetrical appearance with a gap in the bottom left of a funnel plot[26,27].

RESULTS

Overview of included studies

The literature review yielded 1329 abstracts that were screened for inclusion. Of those 1251 were excluded according to our exclusion criteria (see Figure 1) and 78 full articles or meeting abstracts were pulled for full review. Of the 78, 51 were excluded relating to the prevention of AAD (37 were in adult patients, 8 did not provide sufficient diarrhea outcome data, 4 were not associated with antibiotic use or it was unclear if the patients had been exposed to antibiotics, one did not describe the product sufficiently and one was an open dose-ranging study) and 5 articles were also excluded relating to CDI, as they were in adult patients. As a result of the review, 22 pediatric trials were included in this meta-analysis[28-49]. The majority of the trials designated AAD as the primary outcome, while three (14%) trials designated AAD as a secondary outcome[28,41,44].

Of the 22 included clinical trials in pediatric populations, two trials had two treatment arms[34,42] and one trial had two types of controls (a placebo yogurt and a 'no treatment' control)[31]. Erdeve *et al*[34] compared *Saccharomyces boulardii* (*S. boulardii*) to controls using two different antibiotic arms, thus these were analyzed separately. Seki *et al*[42] had two probiotic arms, with *C. butyricum* starting

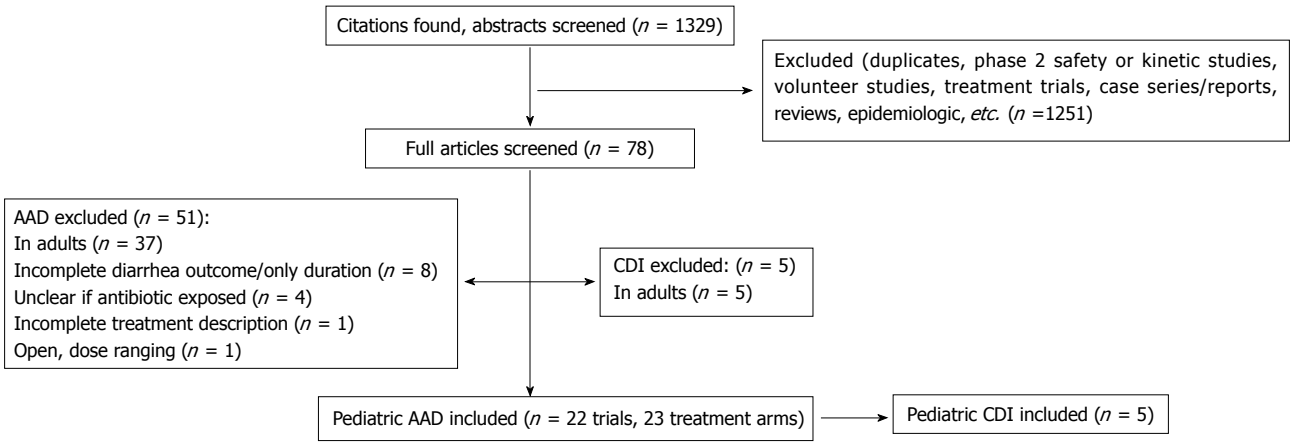

Figure 1 Flow chart of included and excluded trials for pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections. AAD: Antibiotic-associated diarrhea; CDI: *Clostridium difficile* infections.

half-way through the antibiotic exposure and the other arm starting the probiotic from time of antibiotic initiation. As there was no significant difference in the efficacy by the timing of the probiotic, these two arms were combined into one probiotic group. One paper did not present pediatric AAD data separately from adults, and this information was obtained directly from the author[31]. Two controlled trials with uncertain randomization protocols were included to decrease potential publication bias, but were downgraded in their quality score as a result. Four articles were translated from the original language (French, Italian, Persian, or Chinese)[30,37,41,49].

There were no separate randomized controlled trials using probiotics for the prevention of pediatric CDI as their primary outcome, but five trials for the prevention of pediatric AAD included CDI as a secondary outcome in their trial and were thus included[29,33,36,40,43]. We included 22 randomized clinical trials (RCT) evaluating the use of probiotics in a pediatric population for the prevention of AAD and 5 RCT for CDI.

Excluded pediatric studies

Of the 78 articles screened, 42 were in adult populations and were excluded. Of the 14 excluded trials in pediatric populations (Table 1), eight had incomplete documentation of diarrhea outcomes: the outcome was given as days of diarrhea, not AAD incidence[50], outcome was mixed "any GI effects" or "disorders of defection", which grouped diarrhea and/or nausea and/or discomfort[51,52], or no data on diarrhea outcome was reported[53-57]. Four other trials evaluating probiotics in children aimed at the prevention of nosocomial diarrhea were excluded as they either did not document if antibiotic exposure occurred or specifically excluded antibiotic-exposed children[58-61]. One study was excluded as it was an early dose-ranging study, which was not randomized nor used a placebo[62]. One study was excluded as their investigational probiotic also included unknown doses of inulin and lactoferrin[63]. The inter-rater agreement on inclusion and exclusion of trials was 100%.

Patient population

The characteristics of the enrolled study populations by trial arm are presented in Table 2. The age of enrolled pediatric patients ranged from 1 mo to 18 years old and usually included both genders. Race or ethnicity was not reported in most clinical trials. The trials were carried out in a wide array of countries: Poland (*n* = 4), United States (*n* = 3), Finland (*n* = 2), Iran (*n* = 2), China (*n* = 2) and one each in Brazil, Bulgaria, France, Italy, Japan, Philippines, Thailand, Turkey and the United Kingdom. The clinical setting was usually outpatient only (*n* = 11, 50%) or inpatient only (*n* = 6, 27%) or a combination of inpatient and outpatients (*n* = 4, 18%) and the type of practice was not reported in one trial.

The type of infection for which the antibiotic(s) were prescribed included mixed types of infections (respiratory and/or urinary tract and/or otitis media) in 10 trials (45%), or were restricted to one type of infection [respiratory, in 6 trials (27%) or *Helicobacter pylori* (*H. pylori*) in three trials (14%) or otitis media in one trial (4%)] and the type of infection was not reported in two trials, as shown in table 2[34,38].

Antibiotic exposure

Type of antibiotics: Three trials (four treatment arms) limited inclusion due to a single type of antibiotic: amoxicillin[46,47], or sulbactam-ampicillin[34], or azithromycin[34]. Three trials limited antibiotic exposure to the two contained in the standard triple therapy components for *H. pylori* infections (amoxicillin and clarithromycin or furazolidone)[28,41,44]. The majority of trials (*n* = 15, 68%) included a mixture of eligible antibiotic types[29-33,35-37,39,40,42,43,45,48,49] and one trial did not report the type of antibiotic[38]. Most common types of antibiotics were in these mixed-typed antibiotic trials included: amoxicillin (19%-66%), ampicillin (76%), penicillin (47%-71%), cephalosporins (11%-89%).

Duration of antibiotic use: While most trials did not provide the time of antibiotic exposure prior to study, the

Table 1 Excluded studies from pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections by year of publication and reason

Probiotic strain	Outcome	Reason for exclusion	Ref.
C. butyricum	AAD	Open, dose ranging study	Kurata *et al*[62], 1988
L. acidophilus + *Bifido bifidum*	Prevent AAD	Outcome measured by days of diarrhea. No data on AAD incidence	Contardi *et al*[50], 1991
Bifido bifidum _ *Strept thermo*	Prevention of nosocomial diarrhea	Unclear how many were exposed to antibiotics	Saavedra *et al*[58], 1994
L. acidophilus + *L. bulgaricus*	Prevent GI effects	No data on AAD, just "any GI effects"	Witsell *et al*[51], 1995
L. rhamnosus GG	Prevention of nosocomial diarrhea	Unclear how many were exposed to antibiotics	Szajewska *et al*[59], 2001
L. acidophilus + *Bifido infantis* + FOS	Increase in body weight for children on antibiotics	No data on diarrhea provided	Schrezenmeir *et al*[56], 2004
L. casei	Eradicate *H. pylori* in children given triple therapy	No data on diarrhea provided	Sýkora *et al*[57], 2005
L. reuteri	Reduce side effects of triple therapy for pediatric *H. pylori* infections	No data on diarrhea provided, only "disorders of defecation", mixed diarrhea and upper GI	Lionetti *et al*[52], 2006
L. acidophilus + *L. rhamnosus*	Eradicate *H. pylori* in children given triple therapy	No data on diarrhea provided	Plewinska *et al*[55], 2006
Bifido animalis + *L. casei*	Eradication of *H. pylori* in children	Side effects (diarrhea) not documented	Goldman *et al*[53], 2006
S. boulardii	Eradication of *H. pylori* in children	No data on diarrhea provided	Hurduc *et al*[54], 2009
L. rhamnosus GG	Prevention of nosocomial pediatric respiratory or GI infections	None were exposed to antibiotics	Hojsak *et al*[60], 2010
L. reuteri DSM 17938	Prevent nosocomial diarrhea in children	Unclear how many were exposed to antibiotics	Wanke *et al*[61], 2012
Mix of 9 bacterial strains and inulin and lactoferrin	Improve *H. pylori* eradication and reduce AAD in children	Poorly described product (unknown concentrations of inulin and lactoferrin)	Tolone *et al*[63], 2012

S. boulardii: *Saccharomyces boulardii*; *L. rhamnosus*: *Lactobacillus rhamnosus*; *H. pylori*: *Helicobacter pylori*; AAD: Antibiotic-associated diarrhea; CDI: *Clostridium difficile* infection; GI: Gastrointestinal.

trial intervention typically started as soon as possible after the antibiotic was initiated. Overall, the mean duration of antibiotic use during the trial averaged between 7 and 10 d, but the range was broad (3-30 d).

Antibiotic route: Most trials (n = 12, 54%) included children using oral antibiotics[28-31,34,37,41,42,44,46-48]. Mixed intravenous and oral antibiotics were given in six trials[32,36,39,40,45,49]. One trial was limited to solely intravenous antibiotics[43]. In three trials, it was unclear what antibiotic route was used[33,35,38].

Definition of AAD and CDI

Most trials defined AAD as diarrhea associated with the use of antibiotics (any type, route, or duration). The standard definition of AAD in adults, ($\geq$ 3 loose or watery stools per day for $\geq$ 2 consecutive days) was used in 10 (45%) of the trials (as shown in Table 2). Other trials just required either $\geq$ 3[30,42] or $\geq$ 2[34,37,48,49] or $\geq$ 1[46] loose or watery stools per day, but did not require a specific number of days to be considered as defined diarrhea. Less stringent definitions were used by two trials: "parent report"[47] or "otherwise unexplainable diarrhea"[33] and three trials did not report a definition for AAD[35,38,39].

Of 22 trials, only 8 (36%) tested diarrheal stools for viral (adenovirus, rotavirus, calicivirus or astrovirus) and bacterial (*Salmonella, Shigella, Yersinia, Campylobacter, Staphylococcus aureus*, *C. difficile* and *yeasts*) enteric pathogens[28,29,32,36,39,40,43,44]. *C. difficile* was diagnosed using standard enzyme immunoassays (EIA) for toxins A/B in four trials for children who developed diarrhea[29,36,40,43], and in two trials, the type of *C. difficile* assay was not reported[33,42].

Most of the trials (16, 73%) used daily diaries given to the parents or the child to document gastrointestinal symptoms and adverse events, but one trial in inpatient children had hospital staff chart symptoms[35] and one had staff call parents[48], while four trials did not describe the method used to collect gastrointestinal symptoms[33,34,39,42].

Intervention

Details of the intervention for the 22 trials (23 trial arms) are given in Tables 3 and 4.

Randomization: Of the 22 trials, 20 were randomized, but two did not clearly report if they were randomized[39,42]. Seki *et al*[42] only stated "the subjects were divided into three groups" and does not provide a method for randomization, but does provide data showing that the three treatment groups were not significantly different by gender, age, type of antibiotic distribution or treatment group assignment. The other trial was from a published meeting abstract and a full paper was never found in the literature nor were we successful in contacting the authors, which might have provided more details on the methods used[39].

Degree of blinding: Of the 22 trials, 15 (68%) were double-blinded, one was single-blinded and 6 (27%) were open trials (due to the nature of the control group used), as shown in Table 3. One trial used two types of controls, an identical looking and smelling yogurt (double-blinded comparison) and a "no treatment" control arm (open)[31].

Table 2 Characteristics of enrolled pediatric population and probiotic therapies for 22 clinical trials of pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections

Probiotic strain	Age range	Country	Setting (inpatient or outpatient)	Type of inciting infection	Type of antibiotic (s)	Diarrhea defined[1]	Ref.
S. boulardii lyo	1-5 yr	France	Out	Resp	Mixed: amox (19%), ceph (11%)	> 3	Benhamou *et al*[30], 1999
S. boulardii lyo	1-15 yr	Turkey	Out	Nr	Sulbactam-ampicillin only	> 2	Erdeve *et al*[34], 2004
S. boulardii lyo	1-15 yr	Turkey	Out	Nr	Azithromycin only	> 2	Erdeve *et al*[34], 2004
S. boulardii lyo	6 mo-14 yr	Poland	In and Out	OM, Resp	Mixed: ceph (41%), amox (29%)	> 3/2 d	Kotowska *et al*[36], 2005
S. boulardii lyo	6 mo-14 yr	China	In	Resp	IV only: ceph (52%), amox (26%)	> 3/2 d	Shan *et al*[43], 2014
L. rhamnosus GG	5 mo-11 yr	Finland	Out	OM	Amox only	"by parents"	Vaisanen *et al*[47], 1998
L. rhamnosus GG	2 wk-12.8 yr	Finland	Out	Resp	Mixed: amox (66%)	> 3/2 d	Arvola *et al*[29], 1999
L. rhamnosus GG	6 mo-10 yr	United States	Out	Resp, UTI, skin	Mixed: amox (52%)	> 2	Vanderhoof *et al*[48], 1999
L. rhamnosus GG	5-17 yr	Poland	In	*H. pylori* +	Amox and clarithromycin only	> 3/2 d	Szajewska *et al*[44], 2009
L. sporogenes (aka Bacilllus sporogenes) + FOS	4 mo-15 yr	Italy	Out	Resp	Mixed: ceph (41%), amox (30%)	> 2	La Rosa *et al*[37], 2003
C. butyricum MIYAIRI	1 mo-15 yr	Japan	Nr	Resp, GI	Mixed: ceph (48%)	> 3	Seki *et al*[42], 2003
L. acidophilus	1 mo-18 yr	Bulgaria	In	Resp, Pyel	Mixed: b-lactams, clinda, amino	Nr	Pancheva-dimitrova *et al*[39], 2004
B. clausii	6 mo-12 yr	Philippines	In and Out	Resp, GU, Skin	Mixed beta-lactams: pen (47%), ceph (35%)	"otherwise unexplained"	Destura *et al*[33], 2008
Mixes							
L. acidophilus +*L. bulgaricus*	5 mo-6 yr	United States	Out	OM, pharyn, *etc.*	Amox only	> 1	Tankanow *et al*[46], 1990
L. acidophilus + *Bifido infantis*	1-36 mo	Thailand	In	Sepsis or meningitis	Mixed: cefotaxime (89%)	Nr	Jirapinyo *et al*[35], 2002
Bifido lactis + *Strept thermophilus*	6-36 mo	Brazil	In	Nr	Mixed: amp (76%), amox (58%)	> 3/2 d	Corrêa *et al*[32], 2005
Bifido longum PL03 + *L. rhamnosus* KL53A + *L. plantarum* PL02	5 mo-16 yr	Poland	In and Out	Resp, OM, UTI	Mixed: amox (43%), ceph (26%)	> 3/2 d	Szymański *et al*[45], 2008
L. rhamnosus (3 strains) E/N, Pen and Oxy	3 mo-14 yr	Poland	In and Out	Resp, OM, UTI, skin	Mixed: amp or pen (50%), ceph (37%)	> 3/2 d	Ruszczyński *et al*[40], 2008
Kefir (mix of 9 strains)[1]	1-5 yr	United States	Out	Resp	Nr	Nr	Merenstein *et al*[38], 2009
C. butyricum + *Bifido. infantis*	3 mo-3 yr	China	In	Pneumonia	Mixed: ceph (46%)	> 2	Investigating Group for Prevention of AAD in Children with Pneumonia by Clostridium Butyricum and Bifidobacterium[49], 2012
Bifido animalis + *L. acidophilus* + *Strept thermophilus*	1-17 yr	United Kingdom	Out	Resp, skin, UTI	Mixed: pen (71%)	> 3/2 d	Conway *et al*[31], 2007
L. casei + *L. acidophilus* + *L. reuteri* + *L. bulgaricus* + *Strept. cremoris* + *Bifido. bifidum* + *Bifido. infantis* + FOS	4-14 yr	Iran	Out	*H. pylori* +	Amox and clarithromycin only	> 3/2 d	Saneeyan *et al*[41], 2011
L. casei + *L. rhamnosus* + *L. bulgaricus* + *L. acidophilus* + *Strept. thermophilus* + *Bifido. breve* + *Bifido. infantis*	3-14 yr	Iran	Out	*H. pylori* +	Amox and furazolidone only	> 3/2 d	Ahmad *et al*[28], 2013

Diarrhea defined as > 3/2 d: indicates 3 or more loose/watery stools for at least 2 consecutive days; > 3: 3 or more loose or watery stools/day; > 2: 2 or more watery stools/day; "by parents": Defined by parents; "otherwise unexplained": Diarrhea with no other explanation associated with antibiotic use. Amino: Aminoglycosides; Amp: Ampicillin; Amox: Amoxicillin +/- clavulanic acid; Ceph: Cephalosporins; Clinda: Clindamycin; GU: Genito-urinary; FOS: Fructooligosaccharide (990 mg/d); *H. pylori*: *Helicobacter pylori*; In: Inpatient; Nr: Not reported; OM: Otitis media; Out: Outpatient; Pharyn: Pharyngitis; Pyel: Pyelonephritis; Resp: Upper or lower respiratory tract infection; UTI: Urinary tract infection. [1]Kefir contains: *Lactococcus plantarum*, *L. rhamnosus*, *L. acidophilus*, *L. casei*, *L. lactis subspecies diacetylactis*, *Leuconostoc cremoris*, *Bifido. longum*, *Bifido. breve*, *Saccharomyces florentinus*.

Of the 15 double-blinded trials, most (11, 73%) described how treatments were concealed (*e.g.*, identical appearance and taste), but four trials did not provide any further details, other than the trial was double blinded[28,35,46,47].

Table 3 Description of the interventions for 22 clinical trials of pediatric antibiotic-associated diarrhea and *Clostridium difficile*

Probiotic strain	Randomized	Blinding	Type of controls	Formulation	Daily dose (cfu/d)	Duration treatment	Follow-up post-treatment	Ref.
S. boulardii lyo	Yes	Double	Active (diosmectite)	Capsules	4.5×10^9	6-10 d	None	Benhamou *et al*[30], 1999
S. boulardii lyo	Yes	Open	No treatment	Sachet	5×10^9	duration	2 wk	Erdeve *et al*[34], 2004
S. boulardii lyo	Yes	Open	No treatment	Sachet	5×10^9	duration	2 wk	Erdeve *et al*[34], 2004
S. boulardii lyo	Yes	Double	Placebo	Wafers	1×10^{10}	5-7 d	2 wk	Kotowska *et al*[36], 2005
S. boulardii lyo	Yes	Open	No treatment	Powder	1×10^{10}	2 wk	2 wk	Shan *et al*[43], 2014
L. rhamnosus GG	Yes	Double	Milk control	Whey drink	8×10^{10}	7 d	None	Vaisanen *et al*[47], 1998
L. rhamnosus GG	Yes	Double	Placebo	Capsules	4×10^{10}	7-10 d	3 mo	Arvola *et al*[29], 1999
L. rhamnosus GG	Yes	Double	Placebo	Capsules	$1\text{-}2 \times 10^{10}$	10 d	None	Vanderhoof *et al*[48], 1999
L. rhamnosus GG	Yes	Double	Placebo	Capsules	2×10^9	7 d	6 wk	Szajewska *et al*[44], 2009
L. sporogenes [aka Bacilllus sporogenes] + FOS	Yes	Double	Placebo	Capsules	5.5×10^8	10 d	None	La Rosa *et al*[37], 2003
C. butyricum MIYAIRI	Nr	Open	No treatment	Capsules	$1\text{-}4 \times 10^7$	6 d	None	Seki *et al*[42], 2003
L. acidophilus	Nr	Open	No treatment	Nr	2×10^9	duration	None	Pancheva-Dimitrova *et al*[39], 2004
B. clausii	Yes	Open	No treatment	Powder	4×10^9	7-21 d	6 wk	Destura *et al*[33], 2008
Mixes								
L. acidophilus +*L. bulgaricus*	Yes	Double	Placebo	Sachets	2×10^9	10 d	None	Tankanow *et al*[46], 1990
L. acidophilus + *Bifido infantis*	Yes	Double	Placebo	Capsules	6×10^9	7 d	None	Jirapinyo *et al*[35], 2002
Bifido lactis + *Strept thermophilus*	Yes	Double	Placebo	Formula	4×10^8	15 d	15 d	Corrêa *et al*[32], 2005
Bifido longum PL03 + *L. rhamnosus* KL53A + *L. plantarum* PL02	Yes	Double	Placebo	Capsules	2×10^8	3-14 d	2 wk	Szymański *et al*[45], 2008
L. rhamnosus (3 strains) E/N, Pen and Oxy	Yes	Double	Placebo	Capsules	4×10^{10}	3-30 d	2 wk	Ruszczyński *et al*[40], 2008
Kefir (mix of 9 strains)	Yes	Double	Heat-killed drink	Drink	$7\text{-}10 \times 10^9$	10 d	4 d	Merenstein *et al*[38], 2009
C. butyricum + *Bifido. infantis*	Yes	Open	No treatment	Sachet	5×10^9	7 d	None	Investigating Group for Prevention of AAD in Children with Pneumonia by Clostridium Butyricum and Bifidobacterium[49], 2012
Bifido animalis + *L. acidophilus* + *Strept thermophilus*	Yes	Double, open	Control yogurt and no treatment groups	Yogurt	1×10^9	12 d	None	Conway *et al*[31], 2007
L. casei + *L. acidophilus* + *L. reuteri* + *L. bulgaricus* + *Strept. cremoris* + *Bifido. bifidum* + *Bifido. infantis* + FOS	Yes	Single	Placebo	Sachet	1×10^9	14 d	2 wk	Saneeyan *et al*[41], 2011
L. casei + *L. rhamnosus* + *L. bulgaricus* + *L. acidophilus* + *Strept. thermophilus* + *Bifido. breve* + *Bifido. infantis*	Yes	Double	Placebo	Sachet	1×10^9	28 d	4-8 wk	Ahmad *et al*[28], 2013

cfu/d: Colony-forming units/day; Diosmectite: An anti-spasmotic; Duration: Treatment given for the duration of the antibiotic; Nr: Not reported; *S. boulardii*: *Saccharomyces boulardii*; *L. rhamnosus*: *Lactobacillus rhamnosus*.

Type of controls: Of the 22 trials, two studies had two separate control groups. One trial had two control groups (a placebo yogurt and a "no treatment/no yogurt" group)[31]. Another study paired *S. boulardii* and placebo groups for each of two different types of antibiotics[34]. Of the 24 control arms (Table 3), 15 (62%) used a placebo comparison, 8 (33%) used a 'no treatment' control consisting of just the antibiotic used in both groups; one trial compared the probiotic to a standard anti-spasmotic (diosmectite) treatment[30].

Formulation used: Most of the 23 treatment arms used a capsule (9 arms, 39%), while six (26%) used sachets, two trials (9%) used fermented drinks[38,47], and two trials used powder[33,43], as shown in table 3. Less frequent formulations used in single trials included: wafers[36], yogurt[31], infant formula[32], while one trial did not report the type of formulation used[39].

Probiotic used

Type of probiotic strain(s): In the 23 treatment arms, 13 (57%) tested a single strain of probiotic and 10 (43%) had 2-9 strains in their test probiotic treatment. Only two probiotic strains, *S. boulardii* lyo and *Lactobacillus rhamnosus* (*L. rhamnosus*) GG were tested in multiple controlled trials, as shown in Table 3.

Probiotic dose: The daily dose of probiotics varied widely from 10^7 cfu/d to 10^{10} cfu/d, as shown in table 3. The most common daily doses were $1\text{-}6 \times 10^9$/d (54% of trials), while only one trial used 10^7/d[42], three trials used 10^8/d[32,37,45], and seven trials (32%) used a higher

Table 4 Outcomes for 22 clinical trials of pediatric antibiotic-associated diarrhea and *Clostridium difficile* infections trials (total 23 treatment arms)

Probiotic strain	Attrition	ITT or APP	Incidence AAD probiotic	Incidence AAD controls	*P* value (% power)	Incidence CDI probiotic	Incidence CDI controls	*P* value (% power)	Ref.
S. boulardii lyo	21%	APP	25/327 (7.6%)	16/289 (5.5%)	0.29 14%	--	--	--	Benhamou *et al*[30], 1999
S. boulardii lyo + SAM	29%	APP	7/117 (5.7%)	30/117 (25.6%)	< 0.001 98%	--	--	--	Erdeve *et al*[34], 2004
S. boulardii lyo + AZT	29%	APP	7/127 (5.5%)	12/105 (11.4%)	0.15 29%	--	--	--	Erdeve[34], 2004
S. boulardii lyo	8.60%	APP	9/119 (8.0%)	29/127 (23.0%)	0.001 87%	3/119 (2.5)	10/127 (7.9%)	0.09 36%	Kotowska *et al*[36], 2005
S. boulardii lyo	15%	APP	6/139 (4.3%)	28/144 (19.4%)	< 0.001 96%	1/139 (0.7%)	8/144 (5.6%)	0.04 42%	Shan *et al*[43], 2014
L. rhamnosus GG	0%	ITT	6/23 (26%)	8/36 (22%)	0.76 3%	--	--	--	Vaisanen *et al*[47], 1998
L. rhamnosus GG	28.70%	APP	3/61 (5%)	9/58 (16%)	0.07 38%	1/61 (1.6%)	1/58 (1.7%)	1 10%	Arvola *et al*[29], 1999
L. rhamnosus GG	6.90%	APP	7/93 (7.5%)	25/95 (26%)	0.001 90%	--	--	--	Vanderhoof *et al*[48], 1999
L. rhamnosus GG	20%	ITT	2/34 (6%)	6/30 (20%)	0.13 26%	--	--	--	Szajewska[44], 2009
L. sporogenes [Bac. sporogenes] + FOS	18%	ITT	14/48 (29%)	31/50 (62%)	0.001 88%	--	--	--	La Rosa *et al*[37], 2003
C. butyricum MIYAIRI	Nr	Nr	6/86 (7%)	16/27 (59%)	< 0.001 99%	0/86	0/27	--	Seki *et al*[42], 2003
L. acidophilus	Nr	Nr	10/215 (4.6%)	30/139 (21.6%)	< 0.001 99%	--	--	--	Pancheva-Dimitrova *et al*[39], 2004
B. clausii	0%	ITT	3/162 (1.8%)	7/161 (4.3%)	0.22 16%	0/162 (0%)	1/161 (0.6%)	0.5 3%	Destura *et al*[33], 2008
Mixes									
L. acidophilus + *L. bulgaricus*	37%	APP	10/15 (66%)	16/23 (69.5%)	1 3%	--	--	--	Tankanow *et al*[46], 1990
L. acidophilus + *Bifido infantis*	0%	ITT	3/8 (37.5%)	8/10 (80%)	0.14 25%	--	--	--	Jirapinyo *et al*[35], 2002
Bifido lactis + *Strept thermophilus*	7.10%	APP	13/80 (16%)	24/77 (31.2%)	0.04 54%	--	--	--	Corrêa *et al*[32], 2005
Bifido longum PL03 + *L. rhamnosus* KL53A + *L. plantarum* PL02	0%	ITT	1/40 (2.5%)	2/38 (5.3%)	0.61 3%	--	--	--	Szymański *et al*[45], 2008
L. rhamnosus (3 strains) E/N, Pen and Oxy	1.20%	ITT	9/120 (7.5%)	20/120 (17%)	0.046 53%	3/120 (2.5%)	7/120 (5.8%)	0.33 16%	Ruszczyński *et al*[40], 2008
Kefir (mix of 9 strains)	6.40%	ITT	11/61 (18%)	14/64 (21.9%)	0.66 5%	--	--	--	Merenstein *et al*[38], 2009
C. butyricum + *Bifido. infantis*	2.10%	APP	15/193 (7.8%)	30/179 (16.8%)	0.01 70%	--	--	--	Investigating Group for Prevention of AAD in Children with Pneumonia by Clostridium Butyricum and Bifidobacterium[49], 2012
Bifido animalis + *L. acidophilus* + *Strept thermophilus*	12%	ITT	2/48 (4%)	3/34 (9%)	0.64 8%	--	--	--	Conway *et al*[31], 2007
L. casei + *L. acidophilus* + *L. reuteri* + *L. bulgaricus* + *Strept. cremoris* + *Bifido. bifidum* + *Bifido. infantis* + FOS	0%	ITT	3/25 (12%)	13/25 (52%)	0.005 80%	--	--	--	Saneeyan *et al*[41], 2011
L. casei + *L. rhamnosus* + *L. bulgaricus* + *L. acidophilus* + *Strept. thermophilus* + *Bifido. breve* + *Bifido. infantis*	0%	ITT	2/33 (6.1%)	8/33 (24.2%)	0.04 40%	--	--	--	Ahmad *et al*[28], 2013

AAD: Antibiotic-associated diarrhea; CDI: *Clostridium difficile* disease; *S. boulardii*: *Saccharomyces boulardii*; *L. rhamnosus*: *Lactobacillus rhamnosus*; Nr: Not reported.

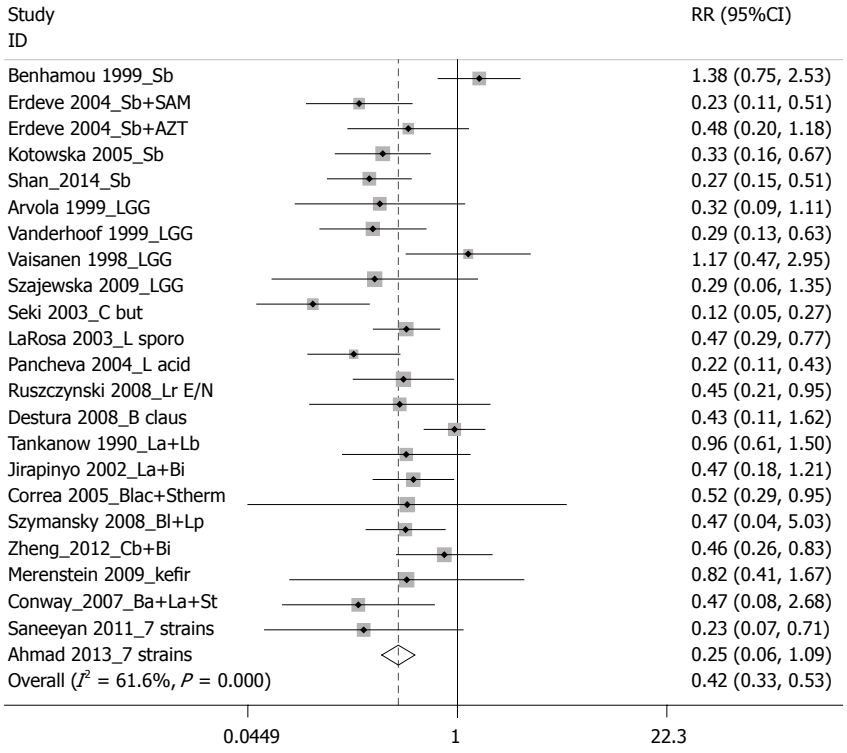

Figure 2 Forest plot of 23 probiotic treatment arms for the prevention of pediatric antibiotic associated diarrhea.

daily dose of probiotic ($\geqslant 10^{10}$/d). As there is no standard recommended dose of probiotics for the pediatric population, doses varied, even for the same strain of tested probiotic. The daily doses in the four trials testing *S. boulardii* lyo ranged from 4.5×10^9 to 1×10^{10} cfu/d. The daily doses in the four trials testing *L. rhamnosus* GG ranged from 2×10^9 to 8×10^{10} cfu/d.

Duration of probiotic treatment: Typically, the probiotic/control treatments are started soon after the inciting antibiotic is begun, but only three trials stated they required the study intervention to begin within 24 h of the antibiotic initiation[35,36,45], while the remaining trials did not specify a minimum time. As the probiotic and control treatment were to be given concurrently with the antibiotic, the time of probiotic/control treatments varied according to the duration of the antibiotic given and ranged from 5 to 30 d, with the most duration of 7-10 d, as shown in Table 3.

Duration of follow-up post-antibiotic: Of the 23 treatment arms, 10 (43%) did not follow the pediatric subjects after the antibiotics and investigational treatments were discontinued. Only four trials followed children for an adequate time (6-12 wk) to capture delayed-onset AAD[28,29,33,44], while nine arms had very short follow-up times, ranging from 4 d to 2 wk, as shown in Table 3.

Attrition: Lost-to-follow up data was reported in 20 (91%) of the 22 trials (Table 4), but was not reported in two trials[39,42]. Six trials (27%) did not report any loss to follow-up[28,33,35,41,45,47], six (27%) had low attrition rates (< 10%)[32,36,38,40,48,49], while eight (36%) had higher attrition rates ranging from 12%-37%[29-31,34,37,43,44,46]. Only 11 (50%) of the trial arms included all enrolled patients in their intent-to-treat analysis, while nine (41%) excluded dropped patients from their as-per-protocol analysis and two (9%) did not report how many dropped from their studies[39,42].

Efficacy of probiotics for AAD

Incidence of pediatric AAD: The incidence of AAD for each treatment arm is presented in table 4. The incidence of AAD in pediatric controls ranged from 4.3%-80%. Of the 23 probiotic treatment arms analyzed separately, 12 (52%) significantly protected children from AAD. As there is significantly heterogeneity in these trials by study size, type of probiotic strain(s) tested, formulation, dose and study design quality, further investigation and analysis was required. A meta-analysis of the 23 treatment arms weighted on study quality score revealed a significant efficacy for probiotics (in general) of a pooled RR for the prevention of pediatric AAD of 0.42 (95%CI: 0.33-0.53), as shown in the forest plot in Figure 2. When the model was run weighted on study size, the pooled results were similar: RR = 0.43 (95%CI: 0.33-0.56, $P < 0.001$). As significant heterogeneity was found ($\chi^2_{22} = 57.4$, $P < 0.001$), a randomized effect model was used in all meta-analysis models. The number needed to treat to prevent one case of pediatric AAD was 8.5.

Incidence of pediatric CDI: The incidence of CDI for each treatment arm analyzed separately is presented in Table 4. Of the five trials, only one significantly protected children from CDI[43]. A meta-analysis of the five treatment arms for the prevention of CDI using probiotics revealed that probiotics are significantly protective for the

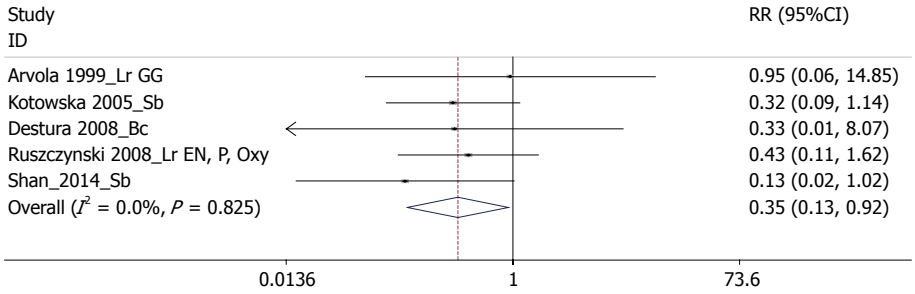

Figure 3 Forest plot of 5 probiotic treatment arms for the prevention of pediatric *Clostridium difficile* disease.

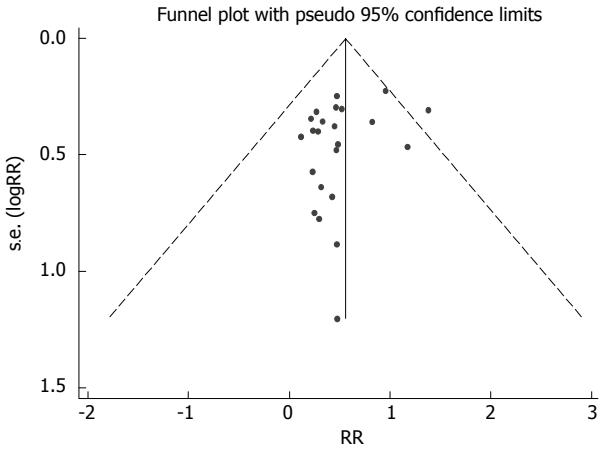

Figure 4 Funnel plot for publication bias assessment from 22 clinical trials for the prevention of pediatric antibiotic associated diarrhea.

prevention of *C. difficile* disease, but only when all strains are pooled (pooled RR = 0.35, 95%CI: 0.13-0.92, P = 0.03), as shown in Figure 3. The number needed to treat to prevent one case of pediatric CDI was 34.8.

Publication bias

A funnel plot analysis (Figure 4) provides no compelling indication of publication bias for AAD trials showing general symmetry of the funnel for the relationship between risk ratio and standard error. Although there are a limited number of trials reporting on the incidence of diarrhea (n = 22), Egger's test for small study effects (P = 0.17) and Begg's test (P = 0.81) also failed to suggest evidence of publication bias. Although our tests for publication bias fail to demonstrate that negative studies remain unpublished, the literature suggests that these tests are, at best, subjective. The only indication that publication bias might exist is the gap in the funnel plot where small studies having an elevated risk for probiotics would appear.

A similar test for publication bias for publication bias for CDI trials also did not indicate significant publication bias (Egger's test, P = 0.62 and Begg's test, P = 0.62), but caution is warranted due to the small number of trials published for pediatric CDI.

Subgroup analysis

Probiotic species: It is well known that not all probiotic strains are equally effective for the prevention of disease, therefore it is necessary to analyze the efficacy by similar probiotic strains whenever possible. Only two probiotic strains have been tested in multiple trials in the pediatric population: *S. boulardii* lyo and *L. rhamnosus* GG. When the five treatment arms (one trial had two treatment arms) testing *S. boulardii* were pooled in a meta-analysis model weighted by study quality[30,34,36,43], there was a significant protective effect for pediatric AAD (pooled RR = 0.43, 95%CI: 0.32-0.60, P < 0.001). When the four trials testing *L. rhamnosus* GG were pooled in a meta-analysis model weighted by study quality[29,44,47,48] this strain is also significantly protective for pediatric AAD (pooled RR = 0.36, 95%CI: 0.19-0.69, P = 0.002).

A meta-analysis for the prevention of CDI was not possible by probiotic strain, as there are no multiple trials within any probiotic strain, other than the two trials for *S. boulardii*.

Probiotic dose: The a *priori* subgroup analyses on dose compared high dose probiotic ($\geqslant 1 \times 10^{10}$ cfu/d) *vs* low dose ($< 1 \times 10^{10}$ cfu/d). Seven of the treatment arms used high daily doses of probiotics and 16 used lower doses (Table 3). For the seven trials using high dose ($\geqslant 1 \times 10^{10}$ cfu/d) probiotics, the pooled incidence of AAD was 8.3% for the probiotic group and 20.6% for the control group (P < 0.001). For the 16 trials using lower doses, the pooled incidence of AAD was 7.3% for the probiotic group and 15.9% for controls (χ^2_1 = 59.3, P < 0.001). A meta-analysis stratifying by low *vs* high dose trials (Figure 5) showed no significant difference by dose (pooled RR by high dose trials, RR = 0.42, 95%CI: 0.31-0.58 and pooled RR by low dose trials, RR = 0.41, 95%CI: 0.30-0.58). If a lower dose threshold was used (5×10^9), there was no significant effect on AAD incidence for probiotics given at 5×10^9 cfu/d (7.2%) *vs* lower doses of probiotics (7.6%). For the 23 different probiotic treatments given, there was no significant dose-effect on the incidence of AAD in children.

Quality of studies: Of the 22 trials, 10 were judged to be of high quality[31,32,36-38,40,43-45,48], 10 trials were judged to be of moderate quality[28-30,33-35,41,42,46,49]. Two trials that had only meeting abstract data available were judged to be of low quality, largely due to missing information[39,47]. The

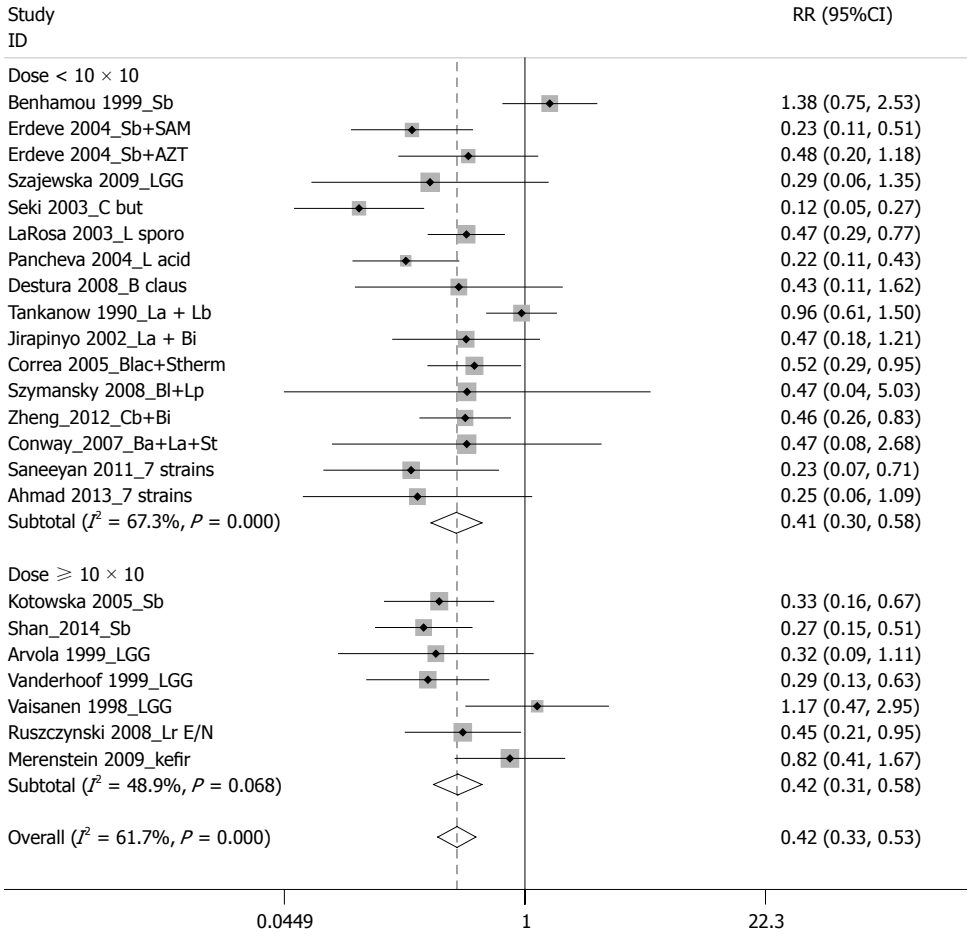

Figure 5 Meta-analysis of pediatric antibiotic-associated diarrhea by high dose (1010 cfu/d) compared to lower doses of daily probiotics given (colony forming units).

33 study items scored on quality were assessed for six sources of potential bias, as shown in Figure 6. Within the study design factors, 82% were scored as high quality, as the studies were typically well described and designed and the interventions were well defined. However, only 36% of trials provided sample size calculations. Within the randomization factors, 64% of the trials were of high quality, but 36% did not describe the method used to generate the randomized treatment allocation numbers. Within the blinding factors, 50% were moderate-low quality, as 32% were not double-blinded and the method of treatment concealment was not well described in 50% of the trials. Within the attrition factors, 41% of the trials were moderate-low quality, and while most (91%) provided attrition rates, only 68% described why children dropped out or were lost-to-follow-up. Within the outcome factors, 54% of the trials were of high quality. Most of the source of reporting bias was due to as-per-protocol analyses (excluding attrition) and not using intent-to-treat analyses. In addition, 27% of the trials did not present a CONSORT flow-chart of the study population and 14% did not present any adverse event data by treatment group. Within the 'other' categories, only 14% of the trials were scored as high quality, largely due to a lack of two topics in the discussion (only 9% discussed generalizability and only 50% discussed limitations of their trial). Other areas that could use improvement were to provide clinical trial registry information and to provide a location where the full protocol may be accessed. The agreement between reviewers on the initial calculation of quality scores was good (kappa = 0.68, 95%CI: 0.63-0.73) and improved after re-review (kappa = 0.98, 95%CI: 0.97-0.99). All disagreements were resolved after further discussion.

Adverse events

Of the 22 trials, 19 (86%) planned a *priori* to document any adverse events that might occur during the intervention and follow-up period (if done), while three trials did not document adverse events during their trials[34,42,47]. None of the trials reported significantly more adverse events in the probiotic group compared to the control groups, nor were there any reported cases of bacteremia or fungemia. Conway *et al*[31] reported 44% abdominal pain and 63% gas in his study, but there was no significant difference by treatment group. La Rosa *et al*[37] reported more (64%) abdominal complaints (cramps, gas and other) in the placebo group than the probiotic group (46%, P = 0.07). Merenstein *et al*[38] reported one case of emesis in the probiotic group and one case of constipation in the

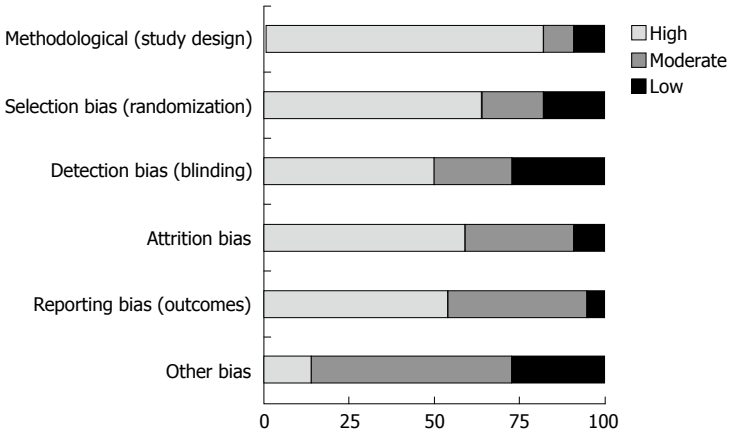

Figure 6 Frequency of study quality based on six different types of potential bias. Low quality: 0%-50% quality items within category not present; Moderate quality: 51%-75% items not present; High quality: 76%-100% items present.

placebo group ($P > 0.05$). Szajewska *et al*[44] reported 18 adverse events in the probiotic group (nausea, vomiting, taste disturbance, loss of appetite, flatulence, constipation), but these were not significantly different than the 13 adverse events reported in the placebo group. Tankanow *et al*[46] reported 14 adverse events (including rash, gas, burping, hiccups, constipation, vomiting, *etc.*), but failed to report in which treatment group these occurred. The result[49] found fewer adverse events in the probiotic group (27%) compared to the placebo group (57%, $P = 0.06$), which included dehydration, fever and vomiting.

GRADE criteria for AAD

For the prevention of pediatric AAD, we recommend the following probiotic strains: *S. boulardii* lyo (high quality and strong strength) and *L. rhamnosus* GG (high quality and strong strength). All other strains require additional multiple randomized, controlled trials before a recommendation can be provided.

GRADE criteria for CDI

For the prevention of pediatric CDI, we are unable to make any recommendations for a specific probiotic strain at the present time due to the limited number of clinical trials performed.

DISCUSSION

Our meta-analyses found that, while in general, probiotics may be an effective strategy to prevent AAD and CDI in children, only a few probiotic strains (*S. boulardii* lyo and *L. rhamnosus* GG) have sufficient evidence from randomized clinical trials to be confident in their abilities to prevent disease in the pediatric population. The safety of probiotics was excellent, as there were no adverse reactions significantly associated with the use of probiotics in any of the 22 clinical trials.

The evidence from meta-analyses of AAD and CDI in the literature have indicated probiotics, in general, may be efficacious for the prevention of AAD or CDI, but two main issues have limited the conclusions for pediatric populations: either trials did not assess the efficacy by specific probiotic strain[13,64] or the authors did not analyze the pediatric data separately[14] or the studies only included adults[65]. Hempel *et al*[13] reviewed 63 randomized controlled trials in adult and pediatric subjects and found a protective effect for probiotics in the prevention of AAD (pooled RR = 0.58, 95%CI: 0.50-0.68), but did not analyze the data by probiotic strain for just pediatric subjects. When the subgroup of pediatric data only was presented, the authors did not present it by probiotic strain. This is an important consideration, as not all probiotics strains are equally effective for AAD or CDI. Two meta-analyses including adult and pediatric subjects did restrict their analysis to trials using only one type of probiotic (*S. boulardii)*, and found a protective effect of this strain (pooled RR = 0.43, 95%CI: 0.23-0.78)[66] and (pooled RR = 0.47, 95%CI: 0.35-0.63)[67], but only one of these 10 trials was in a pediatric population. Kale-Pradhan *et al*[68] reviewed six trials in adults and four trials in children and found the use of different Lactobacilli probiotic strains were protective (RR = 0.35, 95%CI: 0.19-0.67), but not when only pediatric patients were analyzed. Unfortunately, the pediatric data was not analyzed grouped by identical Lactobacilli strain types. Of ten meta-analyses of probiotics for the prevention of AAD found in the literature, only four analyzed probiotics strains separately for pediatric subjects.

Several meta-analyses in pediatric populations only have limited their inclusion to studies to the same probiotic stain for the prevention of AAD. Szajewska *et al*[69] pooled the results from six RCT in children and found *L. rhamnosus* GG was significantly protective in two RCT, but other probiotic strains were not. Johnston *et al*[70-72] also conducted a sub-group analysis by probiotic strains for pediatric cases of AAD over a series of three meta-analysis over time and from the most current meta-analysis of 16 RCT, found *L. rhamnosus* GG was significantly protective in three RCT, but *S. boulardii* did not show a significant efficacy in results pooled from three other RCT. In our meta-analysis, we found both *S. boulardii* and *L. rhamnosus* GG had significant efficacy for preventing pediatric AAD. No other probiotics strains have been tested with multiple clinical trials and this is required before any conclusions and recommendations can be made on other probiotic strains.

There have been several meta-analyses investigating the use of probiotics for the prevention of CDI, but they were in adult populations[65,67,73], or used a pediatric subgroup for the treatment, not prevention, of CDI[74]. Goldenberg *et al*[75] pooled three pediatric trials from their 23 trials in adult and pediatric populations and found a significant protective effect of probiotics for pediatric CDI (RR = 0.37, 95%CI: 0.23-0.60), which was similar to our findings from five randomized controlled trials for CDI (pooled RR = 0.35, 95%CI: 0.14-0.91). Our meta-analysis for the prevention of CDI combined five treatment arms, four with non-significant findings from the individual trials, but overall resulting in a pooled estimate of 65% risk reduction. This finding illustrates a limitation with meta-analytic methods. Although the pooled relative risk indicates a significant protective effect of probiotics, most individual trials did not. This may be interpreted as probiotics, in general, may be an effective strategy for the prevention of pediatric CDI, but the choice of the appropriate tactic (*i.e.* the specific strain of probiotic) has yet to be resolved. As only five randomized clinical trials were found for the prevention of pediatric CDI, but only two trials tested the same strain, we recommend confirmatory clinical trials for these four strains. Clearly, more randomized clinical trials testing specific probiotic strains in multiple trials are required before a conclusion can be reached.

Besides the strain of the probiotic, other factors may either confound the efficacy estimate or be as important as a predictor. These factors may include the dose of probiotic used, the duration used, the formulation and the quality of the study. We investigated the dose of probiotic used and the impact on the efficacy for AAD and CDI by doing sensitivity analyses by different daily doses. Johnston *et al*[72] reported higher dose groups ($\geqslant 5 \times 10^9$ cfu/d) resulted in a significant reduction in pediatric AAD for probiotics (8%) compared to controls (22%) and compared the high dose groups who those developing AAD assigned to lower doses (8% probiotics *vs* 11% in controls), but the apparent dose-effect was driven solely by differences in AAD rates in the control groups, not by rates in the probiotic groups. We did not find a significant dose effect in our meta-analysis of pediatric AAD, as our rates of AAD were similar in the probiotic groups regardless of the threshold used (8% for $\geqslant 10^{10}$ or 7.8% for $\geqslant 5 \times 10^9$ cfu/d and 8.2% for $< 5 \times 10^9$), but our AAD rates did not vary significantly in the control groups depending upon the dose group (21% for $\geqslant 10^{10}$ or 20% for $\geqslant 10^9$ cfu/d and 18% if $< 5 \times 10^9$), unlike the study by Johnston *et al*[72].

The quality of clinical trials varied from a score of 38% to 96%, which was not surprising as some of the trials were done at an earlier time before standardized randomized controlled trial guidelines were widely published and some trials with low quality scores were from meeting abstracts that never resulted in full article publications. The advantage of scoring trials on quality is this allows a meta-analysis model to be run weighing more heavily on higher quality trials. Another advantage of assessing the quality of the clinical trials is the results allow an assessment of recommendations to improve future studies by assessing the different types of bias present in the studies. The trials included in this meta-analysis had generally low rates of bias relating to study design, attrition and reporting bias, but could show improvements in randomization methods and the degree of blinding.

We did not find any significant adverse events associated with the use of probiotics in the 22 pediatric trials and most of the reviews of probiotic clinical trials have not found adverse reactions associated with probiotic use[67]. However, bacteremia and fungemia have been reported in the literature, especially for immunocompromised infants who have a central catheter or have disorders associated with increased bacterial translocation[76-78]. Whelan and Myers reviewed the literature from 1950 to 2009 for adverse reactions noted in trials using probiotics in adult and pediatric populations and found only 20 case reports of adverse events. There were five cases of pediatric bacteremia associated with *L. rhamnosus* GG and six cases of fungemia in children taking *S. boulardii* and all eleven children recovered after treatment with antibiotics or anti-fungals were given[78]. Salminen *et al*[79] reported since the introduction of *L. rhamnosus* GG in Finland in 1990, only 0.02% of blood cultures were positive for Lactobacilli bacteremia and none of the 11 cases were found to have taken the oral probiotic. It is unclear what the absolute risk is for probiotics, as safety data is not routinely collected and reported for children treated with probiotics. The safety of probiotic products is a concern due to the lack of standardized regulations on the quality control of commercial probiotic products and the differing safety regulations depending upon if the probiotic product is an over-the-counter product, dietary supplement or prescribed medication. A review of the field by the World Gastroenterology Organization identified several issues relating to the safety of probiotics, including the inconsistent quality control results due to the failure of some probiotic products to meet their label claims with regard to the numbers and types of viable organisms in their product and the lack of standardization regulations for safety assessment of probiotic products[80]. Sanders *et al*[81] evaluated the safety of probiotic products and also identified several safety concerns: the presence of unlabeled organisms in some of the retail products and the higher rate of sepsis in immunocompromised patients. However, she also found use of probiotics reduced post-surgery infections in five of seven randomized controlled trials[81]. As a consequence, the use of probiotics in severely ill patients should be restricted to probiotic products with strong evidence-based efficacy and beneficial safety profiles.

This systematic review has several strengths. We had specific outcomes selected a *priori* and the search strategy for this review was comprehensive including any relevant trials irrespective of language or publication status (*i.e.* we included published data from meeting abstracts, obtained

pediatric specific data from authors, and translated four non-English trials). Additional strengths of the review include its rigorous application of the GRADE criteria for each of the outcomes[21] and the rigorous evaluation of each of the subgroups (*i.e.* probiotic species, probiotic dose, antibiotic class, and risk of bias) using the 33 criteria for assessing subgroup credibility[82]. The results of this meta-analysis may be generalizable to the global pediatric population, because we included a wide range of ages, countries and settings (inpatients and outpatient children were included). It should be noted however, that ethnicity and race data were not reported, nor were immunocompromised children included in most of the trials, so the applicability of our results to these types of pediatric populations is not known.

This review also has limitations. While we did a more comprehensive search of the grey literature, we did not search all conference proceedings or dissertation abstracts. One of the main limitations for doing meta-analysis on probiotics is the limited number of probiotic strains that have data from multiple trials. Probiotic strain is the key indicator of efficacy for AAD and CDI, but the limited number of trials on the same strain limits our ability draw robust conclusions on most of the strains used for pediatric studies. Only five trials had data on CDI and only two of those were done using the same strain of probiotic. Clearly, more confirmatory research on probiotic strains is mandated. Combining the results of different clinical trials introduces sources of heterogeneity, which may influence the estimate of efficacy. To control for these differences in trial populations and designs, we performed sensitivity analyses by the influence of different doses, by study quality and did separate models for probiotic strains with sufficient numbers of trials. Videlock *et al*[64] used another technique, meta-regression modeling, to assess the association of study-related variables (age, probiotic type, risk of bias and incidence of diarrhea in the placebo group), but failed to find any significant association between these variables and the risk of AAD in adult and pediatric clinical trials. However, it is possible that differences in efficacies found in our meta-analysis may have been influenced by differences in study population, and other study-related variables that we did not stratify on.

The issues of strain-specific efficacy, study design and safety are not unique to the use of probiotics for the prevention of AAD and CDI. Other reviews and meta-analyses of probiotics have also addressed the issues of identifying an appropriate target population, choice of an effective probiotic strain, which needs to be given at an effective dose and for a sufficiently long duration, even though they have been for a different indications than our meta-analysis, such as the treatment of acute pediatric diarrhea[83,84], treatment of adults with irritable bowel syndrome[85,86], and the prevention of adult AAD and CDI[65,67,73].

The alternatives for therapies to prevent pediatric diarrhea are scarce. Racecadotril and diosmectite have been used as adjunctive therapy with oral rehydration therapy in children with existing diarrhea[87], but only diosmectite has been tested in one study for the prevention of diarrhea in pediatric patients receiving pelvic radiation[88]. Thus probiotics remain one of the few strategies available for the prevention of pediatric AAD and CDI.

Suggestions for future research

Recommendations for future research include multiple randomized, controlled trials on the same probiotic strains, allowing confirmation of single clinical trial results. Improvements in study design include reducing bias by the use of treatment concealment (double blinding), calculating sample size a *priori* to power a large enough study to detect significant results, use of intent-to-treat analysis to account for patient attrition effects, the collection of adverse event data and having sufficient follow-up time after the treatments are discontinued. While most cases of AAD occur while a person is on antibiotics; because it takes 6-8 wk for the normal intestinal microbial to become re-established, delayed onset AAD may occur up to 8 wk after antibiotics are discontinued[89,90]. In our meta-analysis, only four of the trials had sufficient follow-up times (6-12 wk) to capture delayed-onset cases of AAD or CDI. Future clinical trials need to incorporate sufficient follow-up times in their study protocols. As the safety of probiotic products continues to be a concern, safety data needs to be collected and global standards for commercial probiotic products are recommended.

In conclusion, our meta-analyses found probiotics are beneficial and safe in the prevention of pediatric AAD and pediatric CDI and, while only two strains had sufficient evidence to conclude they are efficacious for the prevention of AAD (*S. boulardii* lyo and *L. rhamnosus* GG), other probiotic strains are promising.

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COMMENTS

Background

Antibiotic associated diarrhea and *Clostridium difficile* (*C. difficile*) infections are important side-effects of antibiotic use. The frequency of both these diseases is increasing over time and the new therapies are needed to prevent these diseases. The use of probiotics (living organisms that have health benefits) have gained popularity for the prevention and treatment of various diseases, but the evidence can be confusing due to differences in the type of probiotic used and the type of patients treated.

Research frontiers

Of the many available types of probiotic products, only a few have evidence-

based information on the efficacy and safety for the prevention of antibiotic associated diarrhea and *C. difficile* infections. While there are several reviews of probiotics in the adult population, there are limited meta-analysis for the prevention of these two diseases in the pediatric population. The research hot-spot is how to choose the proper probiotic strain(s) for the prevention of these diseases in the pediatric population.

Innovations and breakthroughs

In the present meta-analysis, the largest number of randomized controlled trials in the pediatric population have been reviewed for the efficacy and safety of probiotics. By analyzing the quality of the studies, recommends on how to improve future clinical trials for probiotics have been discovered.

Applications

This meta-analysis found two probiotic strains (*Saccharomyces boulardii* lyo and *Lactobacillus rhamnosus* GG) were found to be significantly preventive for pediatric antibiotic-associated diarrhea. These two probiotics are generally safe to use in pediatric patients, but use is cautioned in children who are immunocompromised or are severely ill.

Terminology

Probiotics are living microorganisms (bacteria or yeasts) that when taken at a sufficient daily dose show a health benefit for the child.

Peer review

This manuscript on the meta-analysis of the use of probiotics in antibiotic-associated diarrhea and *C. difficile* infections is very well written.

REFERENCES

1 **McFarland LV**. Antibiotic-associated diarrhea: epidemiology, trends and treatment. *Future Microbiol* 2008; **3**: 563-578 [PMID: 18811240 DOI: 10.2217/17460913.3.5.563]

2 **McFarland LV**, Brandmarker SA, Guandalini S. Pediatric Clostridium difficile: a phantom menace or clinical reality? *J Pediatr Gastroenterol Nutr* 2000; **31**: 220-231 [PMID: 10997362 DOI: 10.1097/00005176-200009000-00004]

3 **Turck D**, Bernet JP, Marx J, Kempf H, Giard P, Walbaum O, Lacombe A, Rembert F, Toursel F, Bernasconi P, Gottrand F, McFarland LV, Bloch K. Incidence and risk factors of oral antibiotic-associated diarrhea in an outpatient pediatric population. *J Pediatr Gastroenterol Nutr* 2003; **37**: 22-26 [PMID: 12827001 DOI: 10.1097/00005176-200307000-00004]

4 **Zilberberg MD**, Tillotson GS, McDonald C. Clostridium difficile infections among hospitalized children, United States, 1997-2006. *Emerg Infect Dis* 2010; **16**: 604-609 [PMID: 20350373 DOI: 10.3201/eid1604.090680]

5 **Deshpande A**, Pant C, Anderson MP, Donskey CJ, Sferra TJ. Clostridium difficile infection in the hospitalized pediatric population: increasing trend in disease incidence. *Pediatr Infect Dis J* 2013; **32**: 1138-1140 [PMID: 23546535 DOI: 10.1097/INF.0b013e3182948518]

6 **Sammons JS**, Localio R, Xiao R, Coffin SE, Zaoutis T. Clostridium difficile infection is associated with increased risk of death and prolonged hospitalization in children. *Clin Infect Dis* 2013; **57**: 1-8 [PMID: 23532470 DOI: 10.1093/cid/cit155]

7 **Khanna S**, Baddour LM, Huskins WC, Kammer PP, Faubion WA, Zinsmeister AR, Harmsen WS, Pardi DS. The epidemiology of Clostridium difficile infection in children: a population-based study. *Clin Infect Dis* 2013; **56**: 1401-1406 [PMID: 23408679 DOI: 10.1093/cid/cit075]

8 **Kim J**, Shaklee JF, Smathers S, Prasad P, Asti L, Zoltanski J, Dul M, Nerandzic M, Coffin SE, Toltzis P, Zaoutis T. Risk factors and outcomes associated with severe clostridium difficile infection in children. *Pediatr Infect Dis J* 2012; **31**: 134-138 [PMID: 22031485 DOI: 10.1097/INF.0b013e3182352e2c]

9 **Pai S**, Aliyu SH, Enoch DA, Karas JA. Five years experience of Clostridium difficile infection in children at a UK tertiary hospital: proposed criteria for diagnosis and management. *PLoS One* 2012; **7**: e51728 [PMID: 23300561 DOI: 10.1371/journal.pone.0051728]

10 **Schutze GE**, Willoughby RE. Clostridium difficile infection in infants and children. *Pediatrics* 2013; **131**: 196-200 [PMID: 23277317 DOI: 10.1542/peds.2012-2992]

11 **Hoffman FA**, Heimbach JT, Sanders ME, Hibberd PL. Executive summary: scientific and regulatory challenges of development of probiotics as foods and drugs. *Clin Infect Dis* 2008; **46** Suppl 2: S53-S57 [PMID: 18181723 DOI: 10.1086/523342]

12 **Bermudez-Brito M**, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotic mechanisms of action. *Ann Nutr Metab* 2012; **61**: 160-174 [PMID: 23037511 DOI: 10.1159/000342079]

13 **Hempel S**, Newberry SJ, Maher AR, Wang Z, Miles JN, Shanman R, Johnsen B, Shekelle PG. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA* 2012; **307**: 1959-1969 [PMID: 22570464 DOI: 10.1001/jama.2012.3507]

14 **McFarland LV**. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. *Am J Gastroenterol* 2006; **101**: 812-822 [PMID: 16635227 DOI: 10.1111/j.1572-0241.2006.00465.x]

15 **Johnston BC**, Ma SS, Goldenberg JZ, Thorlund K, Vandvik PO, Loeb M, Guyatt GH. Probiotics for the prevention of Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. *Ann Intern Med* 2012; **157**: 878-888 [PMID: 23362517 DOI: 10.7326/0003-4819-157-12-201212180-00563]

16 **Davis LM**, Martínez I, Walter J, Hutkins R. A dose dependent impact of prebiotic galactooligosaccharides on the intestinal microbiota of healthy adults. *Int J Food Microbiol* 2010; **144**: 285-292 [PMID: 21059476 DOI: 10.1016/j.ijfoodmicro.2010.10.007]

17 **Gibson GR**. Dietary modulation of the human gut microflora using prebiotics. *Br J Nutr* 1998; **80**: S209-S212 [PMID: 9924286 DOI: 10.1079/NRR200479]

18 **Surawicz CM**, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. *Am J Gastroenterol* 2013; **108**: 478-498; quiz 499 [PMID: 23439232 DOI: 10.1038/ajg.2013.4]

19 **Moher D**, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Int J Surg* 2012; **10**: 28-55 [PMID: 22036893 DOI: 10.1016/j.ijsu.2011.10.001]

20 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]

21 **Guyatt GH**, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, Schünemann HJ. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008; **336**: 924-926 [PMID: 18436948 DOI: 10.1136/bmj.39489.470347.AD]

22 **Mustafa RA**, Santesso N, Brozek J, Akl EA, Walter SD, Norman G, Kulasegaram M, Christensen R, Guyatt GH, Falck-Ytter Y, Chang S, Murad MH, Vist GE, Lasserson T, Gartlehner G, Shukla V, Sun X, Whittington C, Post PN, Lang E, Thaler K, Kunnamo I, Alenius H, Meerpohl JJ, Alba AC, Nevis IF, Gentles S, Ethier MC, Carrasco-Labra A, Khatib R, Nesrallah G, Kroft J, Selk A, Brignardello-Petersen R, Schünemann HJ. The GRADE approach is reproducible in assessing the quality of evidence of quantitative evidence syntheses. *J Clin Epidemiol* 2013; **66**: 736-742; quiz 742. e1-e5 [PMID: 23623694 DOI: 10.1016/j.jclinepi.2013.02.004]

23 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]

24 **Begg CB**, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**: 1088-1101 [PMID: 7786990 DOI: 10.2307/2533446]

25 **Sutton AJ**, Duval SJ, Tweedie RL, Abrams KR, Jones DR. Empirical assessment of effect of publication bias on meta-

analyses. *BMJ* 2000; **320**: 1574-1577 [PMID: 10845965 DOI: 10.1136/bmj.320.7249.1574]
26 **Sterne JA**, Egger M, Smith GD. Systematic reviews in health care: Investigating and dealing with publication and other biases in meta-analysis. *BMJ* 2001; **323**: 101-105 [PMID: 11451790 DOI: 10.1136/bmj.323.7304.101]
27 **Peters JL**, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. *JAMA* 2006; **295**: 676-680 [PMID: 16467236 DOI: 10.1001/jama.295.6.676]
28 **Ahmad K**, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. *Iran J Pediatr* 2013; **23**: 79-84 [PMID: 23446685]
29 **Arvola T**, Laiho K, Torkkeli S, Mykkänen H, Salminen S, Maunula L, Isolauri E. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. *Pediatrics* 1999; **104**: e64 [PMID: 10545590 DOI: 10.1542/peds.104.5.e64]
30 **Benhamou PH,** Berlier P, Danjou G, Plique O, Jessueld D, DuPont C. Antibiotic-associated diarrhoea in children: A computer monitored double-blind outpatients trial comparing a protective and a probiotic agent. *Med Chir Dig* 1999; **28**: 163-168
31 **Conway S**, Hart A, Clark A, Harvey I. Does eating yogurt prevent antibiotic-associated diarrhoea? A placebo-controlled randomised controlled trial in general practice. *Br J Gen Pract* 2007; **57**: 953-959 [PMID: 18252070 DOI: 10.3399/096016407782604811]
32 **Corrêa NB**, Péret Filho LA, Penna FJ, Lima FM, Nicoli JR. A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants. *J Clin Gastroenterol* 2005; **39**: 385-389 [PMID: 15815206 DOI: 10.1097/01.mcg.0000159217.47419.5b]
33 **Destura RV.** Bacillus clausii in preventing antibiotic-associated diarrhea among Filipino infants and children: A multicenter, randomized, open-label clinical trial of efficacy and safety. Available from: URL: http: //en.sanofi.com/img/content/study/ENTER_L_01125_summary.pdf
34 **Erdeve O**, Tiras U, Dallar Y. The probiotic effect of Saccharomyces boulardii in a pediatric age group. *J Trop Pediatr* 2004; **50**: 234-236 [PMID: 15357564 DOI: 10.1093/tropej/50.4.234]
35 **Jirapinyo P**, Densupsoontorn N, Thamonsiri N, Wongarn R. Prevention of antibiotic-associated diarrhea in infants by probiotics. *J Med Assoc Thai* 2002; **85 Suppl 2**: S739-S742 [PMID: 12403254]
36 **Kotowska M**, Albrecht P, Szajewska H. Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. *Aliment Pharmacol Ther* 2005; **21**: 583-590 [PMID: 15740542 DOI: 10.1111/j.1365-2036.2005.02356.x]
37 **La Rosa M**, Bottaro G, Gulino N, Gambuzza F, Di Forti F, Inì G, Tornambè E. [Prevention of antibiotic-associated diarrhea with Lactobacillus sporogens and fructo-oligosaccharides in children. A multicentric double-blind vs placebo study]. *Minerva Pediatr* 2003; **55**: 447-452 [PMID: 14608267]
38 **Merenstein DJ**, Foster J, D'Amico F. A randomized clinical trial measuring the influence of kefir on antibiotic-associated diarrhea: the measuring the influence of Kefir (MILK) Study. *Arch Pediatr Adolesc Med* 2009; **163**: 750-754 [PMID: 19652108 DOI: 10.1001/archpediatrics.2009.119]
39 **Pancheva-Dimitrova RZ,** Georgieva-Shakola M, Tzaneva V. Probiotics and antibiotic associated diarrhea in children. *Gut* 2004; **53** Suppl 4: A137 (Meeting abstract, 12th Annual Meeting of United European Gastroenterology Week, Prague, 2004
40 **Ruszczyński M**, Radzikowski A, Szajewska H. Clinical trial: effectiveness of Lactobacillus rhamnosus (strains E/N, Oxy and Pen) in the prevention of antibiotic-associated diarrhoea in children. *Aliment Pharmacol Ther* 2008; **28**: 154-161 [PMID: 18410562 DOI: 10.1111/j.1365-2036.2008.03714.x]
41 **Saneeyan H,** Samira L, Hamid R. [Effectiveness of probiotic on treatment of Helicobacter pylori infection in children]. *J Isfahan Med School* 2011; **29**: 882-889
42 **Seki H**, Shiohara M, Matsumura T, Miyagawa N, Tanaka M, Komiyama A, Kurata S. Prevention of antibiotic-associated diarrhea in children by Clostridium butyricum MIYAIRI. *Pediatr Int* 2003; **45**: 86-90 [PMID: 12654076 DOI: 10.1046/j.1442-200X.2003.01671.x]
43 **Shan L,** Hou P, Wang Z, Chen N, Shu L, Zhang H, Han X, Cai X, Shang Y, Vanderplas Y. Prevention and treatment of diarrhea with Saccharomyces boulardii in children with acute lower respiratory tract infections. *Benef Microbes* 2014; in press
44 **Szajewska H**, Albrecht P, Topczewska-Cabanek A. Randomized, double-blind, placebo-controlled trial: effect of lactobacillus GG supplementation on Helicobacter pylori eradication rates and side effects during treatment in children. *J Pediatr Gastroenterol Nutr* 2009; **48**: 431-436 [PMID: 19330931 DOI: 10.1097/MPG.0b013e318182e716]
45 **Szymański H**, Armańska M, Kowalska-Duplaga K, Szajewska H. Bifidobacterium longum PL03, Lactobacillus rhamnosus KL53A, and Lactobacillus plantarum PL02 in the prevention of antibiotic-associated diarrhea in children: a randomized controlled pilot trial. *Digestion* 2008; **78**: 13-17 [PMID: 18701826 DOI: 10.1159/000151300]
46 **Tankanow RM**, Ross MB, Ertel IJ, Dickinson DG, McCormick LS, Garfinkel JF. A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. *DICP* 1990; **24**: 382-384 [PMID: 2109432]
47 **Vaisanen ML,** Leskinen M, Siitonen A, Kanervo A, Harila-Saari A, Mottonen M, Kokkonen J, Rantala H, Saxelin M, Uhari M, Jousimies-Somer H. Occurrence of diarrhea in children receiving oral antibiotics with or without probiotic supplementation with Lactobacillus GG. Meeting abstract from International symposium, PHLS Central Public Health Laboratory, London, 1998. *Microb Ecol in Health Dis* 1998; **10**: 199-200
48 **Vanderhoof JA**, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children. *J Pediatr* 1999; **135**: 564-568 [PMID: 10547243 DOI: 10.1016/S0022-3476(99)70053-3]
49 **Investigating Group for Prevention of AAD in Children with Pneumonia by Clostridium Butyricum and Bifidobacterium.** [Multicenter, randomized, controlled clinical trial on preventing antibiotic-associated diarrhea in children with pneumonia using the live Clostridium butyricum and Bifidobacterium combined Powder]. *Zhonghua Erke Zazhi* 2012; **50**: 732-736 [PMID: 23302558]
50 **Contardi I**. [Oral bacterial therapy in prevention of antibiotic-induced diarrhea in childhood]. *Clin Ter* 1991; **136**: 409-413 [PMID: 1828205]
51 **Witsell DL**, Garrett CG, Yarbrough WG, Dorrestein SP, Drake AF, Weissler MC. Effect of Lactobacillus acidophilus on antibiotic-associated gastrointestinal morbidity: a prospective randomized trial. *J Otolaryngol* 1995; **24**: 230-233 [PMID: 8551535]
52 **Lionetti E**, Miniello VL, Castellaneta SP, Magistá AM, de Canio A, Maurogiovanni G, Ierardi E, Cavallo L, Francavilla R. Lactobacillus reuteri therapy to reduce side-effects during anti-Helicobacter pylori treatment in children: a randomized placebo controlled trial. *Aliment Pharmacol Ther* 2006; **24**: 1461-1468 [PMID: 17032283 DOI: 10.1111/j.1365-2036.2006.03145.x]
53 **Goldman CG**, Barrado DA, Balcarce N, Rua EC, Oshiro M, Calcagno ML, Janjetic M, Fuda J, Weill R, Salgueiro MJ, Valencia ME, Zubillaga MB, Boccio JR. Effect of a probiotic food

as an adjuvant to triple therapy for eradication of Helicobacter pylori infection in children. *Nutrition* 2006; **22**: 984-988 [PMID: 16978844 DOI: 10.1016/j.nut.2006.06.008]

54 **Hurduc V**, Plesca D, Dragomir D, Sajin M, Vandenplas Y. A randomized, open trial evaluating the effect of Saccharomyces boulardii on the eradication rate of Helicobacter pylori infection in children. *Acta Paediatr* 2009; **98**: 127-131 [PMID: 18681892 DOI: 10.1111/j.1651-2227.2008.00977.x]

55 **Plewinska E,** Planeta-Malecka I, Bak-Romaniszyn L, Czkwianianc E, Malecka-Panas E. [Probiotics in the treatment of Helicobacter pylori infection in children.] *Gastroenterol Pol* 2006; **13**: 315-319

56 **Schrezenmeir J**, de Vrese M. Probiotics, prebiotics, and synbiotics--approaching a definition. *Am J Clin Nutr* 2001; **73**: 361S-364S [PMID: 11157342]

57 **Sýkora J**, Valecková K, Amlerová J, Siala K, Dedek P, Watkins S, Varvarovská J, Stozický F, Pazdiora P, Schwarz J. Effects of a specially designed fermented milk product containing probiotic Lactobacillus casei DN-114 001 and the eradication of H. pylori in children: a prospective randomized double-blind study. *J Clin Gastroenterol* 2005; **39**: 692-698 [PMID: 16082279 DOI: 10.1097/01.mcg.0000173855.77191.44]

58 **Saavedra JM**, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 1994; **344**: 1046-1049 [PMID: 7934445 DOI: 10.1016/S0140-6736(94)91708-6]

59 **Szajewska H**, Kotowska M, Mrukowicz JZ, Armańska M, Mikołajczyk W. Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants. *J Pediatr* 2001; **138**: 361-365 [PMID: 11241043 DOI: 10.1067/mpd.2001.11132]

60 **Hojsak I**, Abdović S, Szajewska H, Milosević M, Krznarić Z, Kolacek S. Lactobacillus GG in the prevention of nosocomial gastrointestinal and respiratory tract infections. *Pediatrics* 2010; **125**: e1171-e1177 [PMID: 20403940 DOI: 10.1542/peds.2009-2568]

61 **Wanke M**, Szajewska H. Lack of an effect of Lactobacillus reuteri DSM 17938 in preventing nosocomial diarrhea in children: a randomized, double-blind, placebo-controlled trial. *J Pediatr* 2012; **161**: 40-3.e1 [PMID: 22306046 DOI: 10.1016/j.jpeds.2011.12.049]

62 **Kurata Y,** Taki Y, Inoue K, Miyakawa K. [Preventive effect of live butylic acid bacterium tablet (Miya BM) against diarrhea during administration of antibacterials.] *Jap J Ped Soc* 1988; **41**: 2409-2414

63 **Tolone S**, Pellino V, Vitaliti G, Lanzafame A, Tolone C. Evaluation of Helicobacter Pylori eradication in pediatric patients by triple therapy plus lactoferrin and probiotics compared to triple therapy alone. *Ital J Pediatr* 2012; **38**: 63 [PMID: 23114016 DOI: 10.1186/1824-7288-38-63]

64 **Videlock EJ**, Cremonini F. Meta-analysis: probiotics in antibiotic-associated diarrhoea. *Aliment Pharmacol Ther* 2012; **35**: 1355-1369 [PMID: 22531096 DOI: 10.1111/j.1365-2036.2012.05104.x]

65 **Avadhani A**, Miley H. Probiotics for prevention of antibiotic-associated diarrhea and Clostridium difficile-associated disease in hospitalized adults--a meta-analysis. *J Am Acad Nurse Pract* 2011; **23**: 269-274 [PMID: 21649768 DOI: 10.1111/j.1745-7599.2011.00617]

66 **Szajewska H**, Mrukowicz J. Meta-analysis: non-pathogenic yeast Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. *Aliment Pharmacol Ther* 2005; **22**: 365-372 [PMID: 16128673 DOI: 10.1111/j.1365-2036.2005.02624.x]

67 **McFarland LV**. Systematic review and meta-analysis of Saccharomyces boulardii in adult patients. *World J Gastroenterol* 2010; **16**: 2202-2222 [PMID: 20458757 DOI: 10.3748/wjg.v16.i18.2202]

68 **Kale-Pradhan PB**, Jassal HK, Wilhelm SM. Role of Lactobacillus in the prevention of antibiotic-associated diarrhea: a meta-analysis. *Pharmacotherapy* 2010; **30**: 119-126 [PMID: 20099986 DOI: 10.1592/phco.30.2.119]

69 **Szajewska H**, Ruszczyński M, Radzikowski A. Probiotics in the prevention of antibiotic-associated diarrhea in children: a meta-analysis of randomized controlled trials. *J Pediatr* 2006; **149**: 367-372 [PMID: 16939749 DOI: 10.1016/j.jpeds.2006.04.053]

70 **Johnston BC**, Supina AL, Vohra S. Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized placebo-controlled trials. *CMAJ* 2006; **175**: 377-383 [PMID: 16908901 DOI: 10.1503/cmaj.051603]

71 **Johnston BC**, Supina AL, Ospina M, Vohra S. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2007; (**2**): CD004827 [PMID: 17443557 DOI: 10.1002/14651858.CD004827.pub2]

72 **Johnston BC**, Goldenberg JZ, Vandvik PO, Sun X, Guyatt GH. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2011; (**11**): CD004827 [PMID: 22071814 DOI: 10.1002/14651858.CD004827.pub3]

73 **Johnson S**, Maziade PJ, McFarland LV, Trick W, Donskey C, Currie B, Low DE, Goldstein EJ. Is primary prevention of Clostridium difficile infection possible with specific probiotics? *Int J Infect Dis* 2012; **16**: e786-e792 [PMID: 22863358 DOI: 10.1016/j.ijid.2012.06.005]

74 **Johnston BC**, Shamseer L, da Costa BR, Tsuyuki RT, Vohra S. Measurement issues in trials of pediatric acute diarrheal diseases: a systematic review. *Pediatrics* 2010; **126**: e222-e231 [PMID: 20566617 DOI: 10.1542/peds.2009-3667]

75 **Goldenberg JZ**, Ma SS, Saxton JD, Martzen MR, Vandvik PO, Thorlund K, Guyatt GH, Johnston BC. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. *Cochrane Database Syst Rev* 2013; **5**: CD006095 [PMID: 23728658 DOI: 10.1002/14651858.CD006095]

76 **Boyle RJ**, Robins-Browne RM, Tang ML. Probiotic use in clinical practice: what are the risks? *Am J Clin Nutr* 2006; **83**: 1256-1264; quiz 1256-1264 [PMID: 16762934]

77 **Hennequin C**, Kauffmann-Lacroix C, Jobert A, Viard JP, Ricour C, Jacquemin JL, Berche P. Possible role of catheters in Saccharomyces boulardii fungemia. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 16-20 [PMID: 10706174 DOI: 10.1007/s100960050003]

78 **Whelan K**, Myers CE. Safety of probiotics in patients receiving nutritional support: a systematic review of case reports, randomized controlled trials, and nonrandomized trials. *Am J Clin Nutr* 2010; **91**: 687-703 [PMID: 20089732 DOI: 10.3945/ajcn.2009.28759]

79 **Salminen MK**, Tynkkynen S, Rautelin H, Saxelin M, Vaara M, Ruutu P, Sarna S, Valtonen V, Järvinen A. Lactobacillus bacteremia during a rapid increase in probiotic use of Lactobacillus rhamnosus GG in Finland. *Clin Infect Dis* 2002; **35**: 1155-1160 [PMID: 12410474 DOI: 10.1086/342912]

80 **Guarner F**, Khan AG, Garisch J, Eliakim R, Gangl A, Thomson A, Krabshuis J, Lemair T, Kaufmann P, de Paula JA, Fedorak R, Shanahan F, Sanders ME, Szajewska H, Ramakrishna BS, Karakan T, Kim N. World Gastroenterology Organisation Global Guidelines: probiotics and prebiotics October 2011. *J Clin Gastroenterol* 2012; **46**: 468-481 [PMID: 22688142 DOI: 10.1097/MCG.0b013e3182549092]

81 **Sanders ME**, Akkermans LM, Haller D, Hammerman C, Heimbach J, Hörmannsperger G, Huys G, Levy DD, Lutgendorff F, Mack D, Phothirath P, Solano-Aguilar G, Vaughan E. Safety assessment of probiotics for human use. *Gut Microbes* 2010; **1**: 164-185 [PMID: 21327023 DOI: 10.4161/gmic.1.3.12127]

82 **Sun X**, Briel M, Walter SD, Guyatt GH. Is a subgroup effect believable? Updating criteria to evaluate the credibility of subgroup analyses. *BMJ* 2010; **340**: c117 [PMID: 20354011 DOI: 10.1136/bmj.c117]

83 **Salari P**, Nikfar S, Abdollahi M. A meta-analysis and systematic review on the effect of probiotics in acute diarrhea. *Inflamm Allergy Drug Targets* 2012; **11**: 3-14 [PMID: 22309079 DOI: 10.2174/187152812798889394]

84 **Dinleyici EC**, Eren M, Dogan N, Reyhanioglu S, Yargic ZA, Vandenplas Y. Clinical efficacy of Saccharomyces boulardii or metronidazole in symptomatic children with Blastocystis hominis infection. *Parasitol Res* 2011; **108**: 541-545 [PMID: 20922415 DOI: 10.1007/s00436-010-2095-4]

85 **Hosseini A**, Nikfar S, Abdollahi M. Probiotics use to treat irritable bowel syndrome. *Expert Opin Biol Ther* 2012; **12**: 1323-1334 [PMID: 22897430 DOI: 10.1517/14712598.2012.707179]

86 **McFarland LV**, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. *World J Gastroenterol* 2008; **14**: 2650-2661 [PMID: 18461650 DOI: 10.3748/wjg.14.2650]

87 **Faure C**. Role of antidiarrhoeal drugs as adjunctive therapies for acute diarrhoea in children. *Int J Pediatr* 2013; **2013**: 612403 [PMID: 23533446 DOI: 10.1155/2013/612403]

88 **Santantonio M**, Colella M, Fiorica F, Pratissoli S, Stefanelli A, Falchi AM, Venturi A, Balli M. Diosmectite (DIOSMECTAL) in preventive anti-diarrheic therapy in patients subjected to pelvic radiation. *Minerva Gastroenterol Dietol* 2000; **46**: 225-230 [PMID: 16501441]

89 **Dethlefsen L**, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008; **6**: e280 [PMID: 19018661 DOI: 10.1371/journal.pbio.0060280]

90 **McFarland LV**. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. *Dig Dis* 1998; **16**: 292-307 [PMID: 9892789 DOI: 10.1159/000016879]

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META-ANALYSIS

Meta-analysis of anti-ribosomal P antibodies in lupus psychosis

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Abstract

AIM: To perform a meta-analysis of the prevalence of anti-ribosomal P (aRP) antibodies in lupus psychosis, and the odds of psychosis in aRP-positive subjects.

METHODS: We identified articles by searching PubMed, PsychInfo, and ISI, and the reference lists of identified studies.

RESULTS: Twenty-four studies met the inclusion criteria. Positive aRP antibodies were found in 51% (91 of 179 total cases) of cases of lupus psychosis. There was an almost 3.5-fold increased odds of psychosis in aRP-positive patients (OR = 3.46, 95%CI: 1.97-6.09, P < 0.001). The population attributable risk percentage was 36% for aRP antibodies.

CONCLUSION: aRP antibodies are common in lupus psychosis, although the potential mechanism(s) underlying this association remain unclear. Given the overlap between the clinical presentation and risk factors for lupus psychosis and schizophrenia, further investigation of aRP antibodies in schizophrenia is warranted.

Key words: Systemic lupus erythematosus; Psychosis; Autoantibodies; Anti-ribosomal P antibodies; Meta-analysis

Core tip: In a meta-analysis of twenty-four studies, positive anti-ribosomal P (aRP) antibodies were found in 51% (91 of 179 total cases) of cases of lupus psychosis. There was an almost 3.5-fold increased odds of psychosis in aRP-positive patients (OR = 3.46, 95%CI: 1.97-6.09, P < 0.001). The population attributable risk percentage was 36% for aRP antibodies. aRP antibodies are common in lupus psychosis, although the potential mechanism(s) underlying this association remain unclear. Given the overlap between the clinical presentation and risk factors for lupus psychosis and schizophrenia, further investigation of aRP antibodies in schizophrenia is warranted.

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INTRODUCTION

Neuropsychiatric manifestations occur in about half of patients with systemic lupus erythematosus (SLE)[1]. Psychosis is a rare, but well-documented neuropsychiatric sequelae of SLE. A systematic review of 9 studies, comprised of 1422 subjects with SLE, found a 5% point prevalence of lupus psychosis[2]. Lupus psychosis is de-

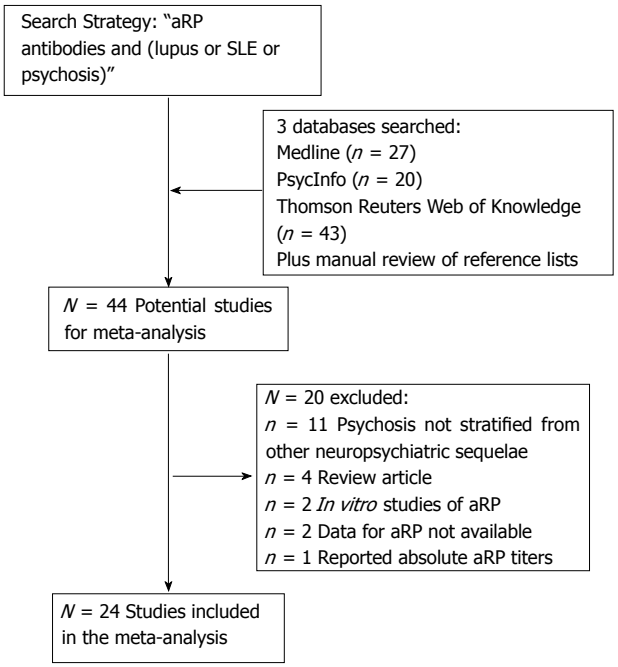

Figure 1 Flowchart of the study selection process. aRP: Anti-ribosomal P.

fined as a severe disturbance in the perception of reality characterized by delusions and/or hallucinations[3]. The diagnostic criteria require that the disturbance (1) includes either delusions or hallucinations without insight; (2) causes clinical distress or impairment in social, occupational, or other relevant areas of functioning; (3) does not occur exclusively during the course of a delirium; and (4) is not better accounted for by another mental disorder. A primary psychotic disorder unrelated to SLE (*e.g.,* schizophrenia), substance- or drug-induced psychosis, and a psychologically medicated reaction to SLE (*e.g.,* brief reactive psychosis with a major stressor) are exclusionary to the diagnosis of lupus psychosis.

The clinical presentation of lupus psychosis may mimic that of schizophrenia. Schizophrenia is a heterogeneous psychotic disorder that requires the presence of two or more characteristic symptoms-delusions, hallucinations, disorganized speech, grossly abnormal psychomotor behavior (such as catatonia), or negative symptoms (*i.e.* restricted affect or avolition/asociality[4]. At least one of these two characteristic symptoms should include delusions, hallucinations, or disorganized speech. There is significant impairment in one or more major areas of functioning, including work, interpersonal relations, and self-care. It must also be established that the disturbance is not better accounted for by a primary mood disorder, schizoaffective disorder, substance intoxication or withdrawal, or another general medical condition.

A recent study found that 2 of 85 subjects hospitalized for a first-episode of schizophrenia had positive anti-nuclear (ANA) antibody titers and subsequently were found to have neuropsychiatric SLE[5]. Importantly, neither subject had signs or symptoms suggestive of rheumatologic disease, and presented only with psychiatric complaints.

A number of previous studies have found an association between anti-ribosomal P (aRP) antibodies and lupus psychosis[6-11]. aRP antibodies target P0, P1 and P2 proteins on the ribosomal sub-unit, and are capable of penetrating cells and inducing apoptotic changes. A previous meta-analysis investigated the accuracy of aRP antibody testing for the diagnosis of neuropsychiatric SLE[1]; however, this study did not consider psychosis separately from other neuropsychiatric manifestations of SLE, such as mood disorders and seizures. The purpose of the present study was to perform a meta-analysis of the prevalence of aRP antibodies in lupus psychosis, and the odds of psychosis in aRP-positive subjects.

MATERIALS AND METHODS

Study design

Studies of aRP antibodies in lupus psychosis were systematically searched using using Medline (PubMed, National Center for Biotechnology Information, United States National Library of Medicine, Bethesda, Maryland), PsycInfo (*via* Ovid, United States Psychological Association, Washington, DC), and Thomson Reuters (formerly ISI) Web of Knowledge (Science Citation Index and Social Sciences Citation Index, Thomson Reuters, Charlottesville, Virginia) in October 2011 and again in March 2013. The primary search strategy was "anti-ribosomal P antibodies and (lupus or SLE or psychosis)." Limiting results to studies in English, this search resulted in 27 citations from Medline, 20 from PsychInfo, and 43 from ISI. From these citations, as well as a manual review of their reference lists, we identified 44 potential studies, which are described in Table 1[1,6-48].

The inclusion criteria were (1) cross-sectional studies of the proportion of subjects with SLE positive for aRP antibodies, stratified by the presence or absence of psychosis; (2) cross-sectional studies of the proportion of subjects with lupus psychosis and positive aRP antibodies; or (3) longitudinal studies of aRP antibodies at multiple time points in subjects with lupus psychosis. The exclusion criteria were: (1) studies in which there were no cases of lupus psychosis; (2) studies which did not stratify psychosis from other neuropsychiatric sequelae of SLE, such as seizures and mood disorders; and (3) *in vitro* studies of aRP antibodies.

After independent searches, review of the study methods by two authors (BJM and KL) 24 studies met the inclusion criteria. There was universal agreement on the independent studies. 20 studies were excluded due: psychosis not stratified from other neuropsychiatric sequelae (n = 11), review articles (n = 4), *in vitro* studies of aRP (n = 2), data for aRP not available (n = 2), and reported absolute aRP titers (n = 1). A flow chart summarizing the study selection process is presented in Figure 1. For each of the 16 case-control studies identified, we also extracted descriptive data on subject age, gender, and illness duration.

Statistical analysis (prevalence of anti-ribosomal P antibodies)

For all 24 studies, we calculated the prevalence of positive

Table 1 Studies of anti-ribosomal P antibodies in systemic lupus erythematosus

Study	Assay method	Location	Included	Comment
Abdel-Nesser 2008	ELISA	Egypt	Yes	Case-control study
Almeida 2002	ELISA	Spain	Yes	
Arnett 1996	ELISA	United States	Yes	Case-control study
Bonfa 1987	Immunoblotting, RIA	United States	Yes	
Briani 2009	Immunoblotting	Italy	Yes	Case-control study
Caponi 2002	ELISA	Italy	Yes	Case-control study
Chan 1998	ELISA, Western blot	China	No	Psychosis not stratified from other neuropsychiatric sequelae
Conti 2004	ELISA	Italy	No	Psychosis not stratified from other neuropsychiatric sequelae
Derksen 1990	ELISA	Netherlands	Yes	
Ebert 2005	N/A		No	Review article
Ghirardello 2001	N/A		No	Review article
Haddouk 2009	Immunodot assay	Tunisia	Yes	Case-control study
Hanly 2008	ELISA	Canada	Yes	Case-control study
Hanly 2011	ELISA, Lupus anticoagulant	Canada	Yes	Case-control study
Hoffman 2004	N/A	Europe	No	Data for aRP not available
Isshi 1996	ELISA	Japan	Yes	Case-control study
Isshi 1998	ELISA	Japan	No	Reported absolute titers
Jonsen 2003	Immunoassays	Sweden	Yes	
Kao 1999	N/A	China	Yes	
Karassa 2005	N/A	Multicenter	No	Psychosis not stratified from other neuropsychiatric sequelae
Magalhaes 2007	ELISA	Brazil	No	Psychosis not stratified from other neuropsychiatric sequelae
Mahler 2003	Indirect immunofluorescence		No	Review article
Massardo 2002	Double immune diffusion, or Western blot and ELISA	Chile	Yes	Case-control study
Munoz 1999	N/A	Spain	Yes	
Nagai 2005	Flow cytometery	Japan	No	In vitro study
Nagai 2011	ELISA	Japan	No	In vitro study
Nojima 1992	western blot	Japan	Yes	Case-control study
Press 1996	ELISA	Canada	Yes	Case-control study
Sanna 2000	N/A	Italy	No	Data for aRP not available
Sato 1991	FIEA	Japan	No	Psychosis not stratified from other neuropsychiatric sequelae
Schneebaum 1991	ELISA	United States	Yes	Case-control study
Shovman 2006	ELISA	Israel	Yes	Case-control study
Teh 1992	ELISA	United Kingdom	Yes	Case-control study
Teh 1993	ELISA	United Kingdom	No	Psychosis not stratified from other neuropsychiatric sequelae
Teh 1993b	ELISA	United Kingdom	No	Psychosis not stratified from other neuropsychiatric sequelae
Toubi 2007	N/A		No	Review article
Tziousfas 2000	Western blot	Isreal	No	Psychosis not stratified from other neuropsychiatric sequelae
Van Dam 1991	ELISA, Immunoblotting	Netherlands	Yes	Case-control study
Watanabe 1996	ELISA	Japan	No	Psychosis not stratified from other neuropsychiatric sequelae
Weiner 2000	Not specified	Germany	No	Psychosis not stratified from other neuropsychiatric sequelae
West 1995	ELISA	United States	Yes	
Williams 2004	Western blot	United States	Yes	
Yalaoui 2002	N/A	Tunisia	No	Psychosis not stratified from other neuropsychiatric sequelae
Yoshio 1995	ELISA	Japan	Yes	Case-control study

aRP: Anti-ribosomal P; FIEA: Fluro-immuno-enzymatic assay; RIA: Radioimmunoassay.

aRP antibodies in lupus psychosis by dividing the number of subjects with psychosis and positive aRP antibodies by the total number of subjects with psychosis.

Meta-analysis

For each of the 16 case-control studies, we calculated odds ratios (OR) and 95% confidence intervals (95%CIs) for psychosis in aRP-positive patients, with odds set equal to 1.00 for psychosis in aRP-negative patients. We then performed a meta-analysis to estimate pooled OR (and 95%CI) for psychosis in aRP-positive patients, again with risk = 1.00 for psychosis in aRP-negative patients. Random effects pooled estimates and 95%CI were calculated using the method of DerSimion and Laird. Random effects models yield their actual first error rate while fixed effect models tend to inflate their first error rate. CIs obtained by fixed effect models are also biased and their actual coverage rate is smaller than their nominal coverage rate[49]. *P*-values were considered statistically significant at the χ^2 = 0.05 level. A funnel plot and Egger's test were generated to assess for publication bias. In case of significant heterogeneity in the overall result, we performed subgroup analysis and meta-regression, to explore possible reasons for the heterogeneity. The subgroup analysis included assay methodology (ELISA *vs* other). We conducted meta-regression analyses of four variables, year of publication, age, the proportion of female subjects, and illness duration. The statistical analyses were performed

Table 2 Effect of anti-ribosomal P antibody status on psychosis risk

Study	Total (*N*)	Mean age (yr)	Female (%)	Mean illness duration (yr)	aRP(+) (*n*)	aRP(-) (*n*)	Psychosis (*n*)	Psychosis and aRP(+) (*n*)	No psychosis and aRP(+) (*n*)	Psychosis and aRP(-) (*n*)	No psychosis and aRP(-) (*n*)	OR	95%CI
Abdel-Nesser 2008	32	25.0	87.5	3.9	7	25	1	1	6	0	25	8.33	0.25-278.68
Arnett 1996	364				63	301	17	8	55	9	292	4.72	1.74-12.76
Briani 2009	219	28.0	84.5		45	174	1	1	44	0	174	7.91	0.26-239.58
Caponi 2002	149	37.1	93.3	9.3	18	131	1	0	18	1	130	0.07	0-2.6 × 10^7
Haddouk 2009	200	30.5	86.5		47	153	3	1	46	2	151	1.64	0.15-18.51
Hanly 2008	214	34.9	87.4	0.4	17	197	7	3	14	4	193	10.34	2.10-50.82
Hanly 2011	991	35.2	89.1	0.5	91	900	14	4	87	10	890	4.09	1.26-13.32
Isshi 1996	75				21	54	19	10	11	9	45	4.55	1.49-13.88
Massardo 2002	141	33.0	90.1	5.0	21	120	2	2	19	0	120	25.26	1.10-581.69
Nojima 1992	91		80.0		38	53	10	9	29	1	53	16.45	1.98-136.36
Press 1996	79				16	63	13	5	11	8	55	3.13	0.86-11.37
Schneebaum 1991	269				51	218	29	13	38	16	202	4.32	1.92-9.71
Shovman 2006	44				6	38	1	1	5	0	38	15.2	0.45-513.80
The 1992	116				18	98	13	3	15	10	88	1.76	0.43-7.15
Van Dam 1991	38				12	26	1	1	11	0	26	4.73	0.15-151.50
Yoshio 1995	70	31.9	94.3		41	29	10	3	38	7	22	0.25	0.06-1.06
Total	3093				512	2581	142	65	447	77	2504		

aRP: Anti-ribosomal P; OR: Odds ratios; CI: Confidence interval.

using Stata 10.0 (StataCorp LP, College Station, TX). The meta-analysis procedure also calculates a χ^2 value for the heterogeneity in effect size (ES) estimates, which is based on Cochran's Q-statistic[50]. Between-study heterogeneity χ^2 was considered significant for $P < 0.10$[51].

RESULTS

Prevalence of anti-ribosomal P antibodies

Positive aRP antibodies were found in 51% (91 of 179 total cases) of cases of lupus psychosis.

Meta-analysis

As described in Table 2, the case-control studies included a total of 3093 subjects. Table 2 and Figure 2 present the estimates of OR with 95%CIs from the meta-analysis. There was an almost 3.5-fold increased odds of psychosis in aRP-positive patients (OR = 3.46, 95%CI: 1.97-6.09, $P < 0.001$). There was significant heterogeneity in this effect size estimate, $\chi^2 = 26.43$, $P = 0.03$. In a post-hoc sensitivity analysis, the heterogeneity was no longer significant ($\chi^2 = 12.63$, $P = 0.55$) and the association was stronger (OR = 4.29, 95%CI: 2.90-6.36, $P < 0.001$) after excluding one study (Yoshio). A funnel plot showed no evidence of publication bias (Figure 3; Eggers test, $P = 0.99$).

In the subgroup analysis, there was no change in the association when studies using ELISA to measure aRP antibodies were considered separately (OR = 3.00, 95%CI: 1.60-5.59, $P < 0.001$). In meta-regression analyses, year of publication ($P = 0.55$), age (0.55), and illness duration ($P = 0.27$) were unrelated to the association between aRP antibodies and lupus psychosis. However, there was a significant association with gender (slope = -0.31, 95%CI: -0.55 to -0.08, $P = 0.02$), with a stronger association in studies with a higher proportion of males (Figure 4).

We also estimated the population attributable risk percentage (PAR%) for aRP-positivity. The PAR% is the prevalence of the outcome (psychosis) in all subjects, minus the prevalence of the outcome among the unexposed (defined here as aRP negative patients), divided by the prevalence of outcome in the total population, and multiplied by 100%. The population PAR% was 36% for aRP antibodies.

DISCUSSION

Although psychosis is a rare neuropsychiatric manifestation of SLE, we found that more than half of subjects with lupus psychosis had positive aRP antibodies. Furthermore, there was an almost 3.5-fold increased odds of psychosis in aRP-positive patients. The association was not moderated by year of publication, age, or illness duration, but there was a significant association with gender. The PAR% was 36% for anti-ribosomal P antibodies.

An important strength of our study is that we included data from all case-control studies of this association. A previous meta-analysis aRP antibodies in SLE did not consider psychosis separately from other neuropsychiatric manifestations of SLE[1]. Our analysis differed from this study in several ways. First, we focused on psychosis as the outcome, rather than the broader category of neuropsychiatric SLE. Second, we were able to calculate the odds of psychosis in aRP-positive subjects, as well as the PAR% for aRP-positivity. Although we were able to perform subgroup and meta-regression analyses, an important limitation of the present study was that data on a number of potential confounding factors, including age, sex, and illness duration, were available for only a portion of studies. We were not able to control for other potential confounding factors including smoking status,

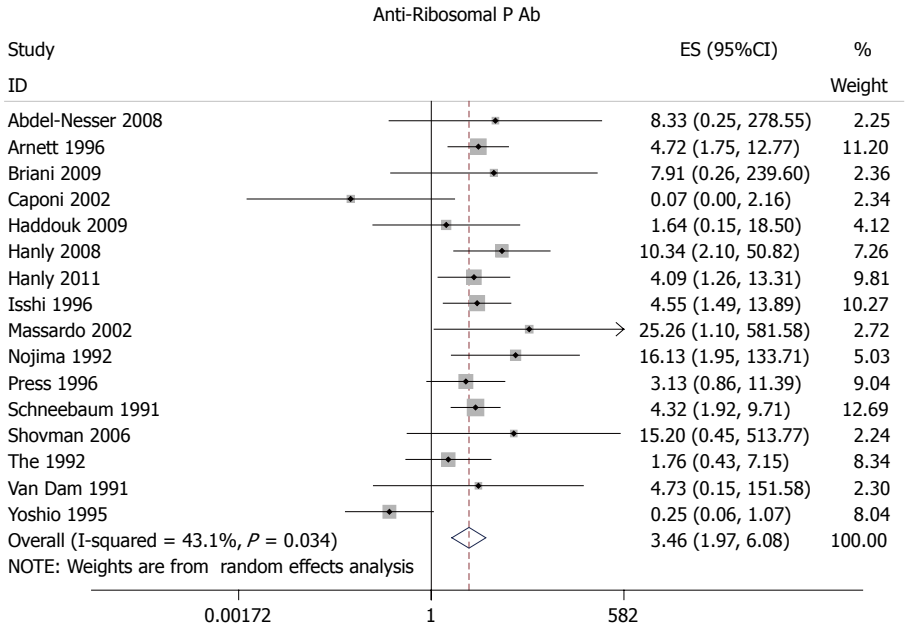

Figure 2 Forest plot of risk of psychosis in anti-ribosomal P antibody-positive subjects.

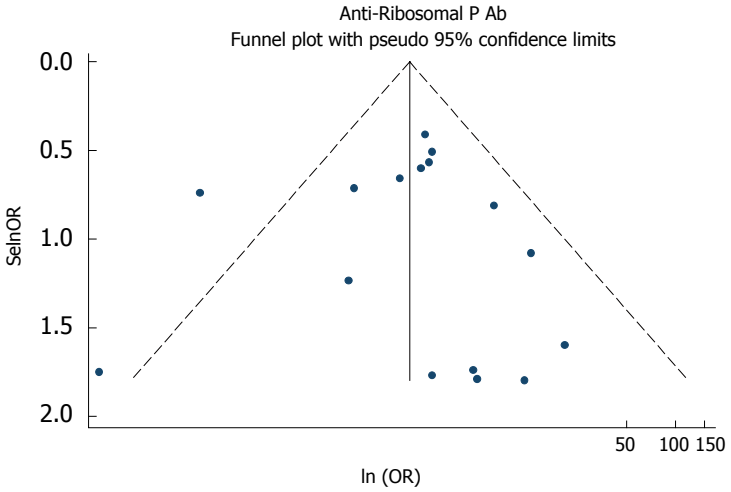

Figure 3 Funnel plot of studies of anti-ribosomal P antibodies in lupus psychosis.

rheumatologic symptoms, stage of illness (*e.g.,* active *vs* inactive SLE), and medications.

We found a population attributable risk percentage (PAR%) of 36% for aRP-positivity. As the PAR% varies with both the risk (*i.e.* OR) associated with an exposure (*i.e.* aRP-positivity) and its prevalence, caution must be exercised in the interpretation of this result. The PAR% refers to a family of concepts. Greenland and Robins[52] distinguished between the etiologic and excess fraction. The etiologic fraction is the proportion of cases that the exposure had played a causal role in its development. The excess fraction is the proportion of cases among the exposed population that is in excess in comparison with the unexposed. Our results describe the excess fraction for aRP-positivity, as it is not possible to establish the causality of this association.

One longitudinal study found that IgA and IgM classes of aRP antibodies were elevated at the onset of psychosis, and titers decreased following a remission of psychosis[43]. Another longitudinal study of aRP activity in two patients with psychosis revealed that aRP levels increased before and during the active phases of psychosis[15]. This could possibly help predict efficacy of treatment and warrants further investigation into the possibility of monitoring disease activity by aRP titers.

The mechanism(s) underlying this association remain unclear and warrant further investigation. One possibility is that aRP antibodies may directly cross-react with central nervous system antigens, resulting in acute psychosis. Autoantibodies are also associated with increases in pro-inflammatory cytokines, such as interleukin-6 (IL-6) which can directly modulate dopaminergic neurotransmission[53], or indirectly modulate glutamatergic neurotransmission through tryptophan catabolism[54], which can also result in acute psychosis. Consistent with the latter, increased cerebrospinal fluid IL-6 is also associated with lupus psychosis, although the relationship with aRP antibodies is unknown[55].

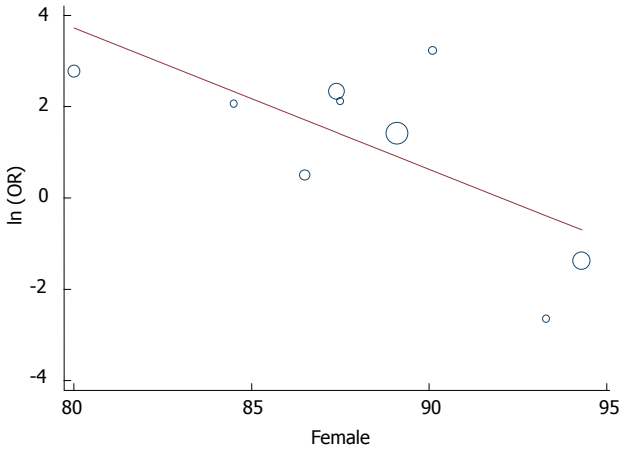

Figure 4 Meta-regression analysis of the effect of the proportion of female subjects on the association between anti-ribosomal P antibodies and psychosis.

A previous study found 2 of 85 subjects presenting with first-episode schizophrenia were subsequently diagnosed with neuropsychiatric SLE, and neither subject had other signs or symptoms of rheumatologic disease[5]. A systematic quantitative review also found an increased prevalence of autoantibodies associated with limbic encephalitis (NMDA receptor antibodies) in subjects with first-episode schizophrenia, in the absence of other neurologic signs or symptoms[56]. To our knowledge, only one previous study has measured aRP antibodies in subjects with schizophrenia[57]. Among 59 patients in this study, aRP antibody titers were below cutoff levels in 58 patients and borderline in 1 patient. One possibility for the negative finding is that the prevalence of potentially pathogenic central nervous system autoantibodies in schizophrenia is low, and this study was underpowered to detect an association. Another possibility is that serum autoantibodies are only present earlier in the course of the disorder.

In addition to overlapping clinical presentations, there are also shared risk factors for lupus psychosis and schizophrenia. There is bidirectional evidence for an association between schizophrenia and autoimmune disorders[58-60]. Single nucleotide polymorphisms in genes in the major histocompatibility complex on chromosome 6q, which are critical to immune system function and associated with autoimmune disorders, are also risk factors for schizophrenia[61-63]. Patients with schizophrenia may also have abnormal absolute levels of antibody-producing B-lymphocytes[64-66]. Thus, as identification of patients with autoantibody-mediated psychosis (*vs* schizophrenia) has important treatment-related implications, these findings suggest that future studies of aRP antibodies in patients with schizophrenia are warranted.

In conclusion, aRP antibodies are highly prevalent and significant predictors of lupus psychosis. Future studies of these antibodies will be important to an improved understanding of the pathophysiology of psychosis. Further investigation of these autoantibodies in patients with schizophrenia, which has largely been unexplored, are warranted.

ACKNOWLEDGEMENTS

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COMMENTS

Background

Neuropsychiatric manifestations occur in about half of patients with systemic lupus erythematosus (SLE). Psychosis is a rare, but well-documented neuropsychiatric sequelae of SLE. A number of previous studies have reported an association between anti-ribosomal P (aRP) antibodies and lupus psychosis.

Research frontiers

The purpose of the present study was to perform a meta-analysis of the prevalence of aRP antibodies in lupus psychosis, and the odds of psychosis in aRP-positive subjects.

Innovations and breakthroughs

A previous meta-analysis investigated the accuracy of aRP antibody testing for the diagnosis of neuropsychiatric SLE; however, this study did not consider psychosis separately from other neuropsychiatric manifestations of SLE, such as mood disorders and seizures. In a meta-analysis of 24 studies, we report that positive aRP antibodies were found in 51% (91 of 179 total cases) of cases of lupus psychosis. There was an almost 4-fold increased odds of psychosis in aRP-positive patients (OR = 3.75, 95%CI: 2.23-6.30, $P < 0.001$). The population attributable risk percentage was 36% for aRP antibodies.

Applications

aRP antibodies are common in lupus psychosis. Schizophrenia is associated with increased prevalence of autoantibodies and autoimmune disease. Given these associations, aRP warrants further investigation in schizophrenia.

Terminology

aRP antibodies: Autoantibodies are immune molecules (proteins) that are directed against the body's own tissues. aRP antibodies target proteins on the ribosome, and are capable of penetrating cells and inducing apoptosis, or programmed cell death.

Psychosis: Psychosis is a potential neuropsychiatric complication that occurs in some patients with systemic lupus erythematosus. Psychosis is a neuropsychiatric disorder that includes abnormalities in thinking, behavior, mood, and cognition. Common symptoms of psychosis included hallucinations, delusions, disorganized speech and behavior, and negative symptoms.

Peer review

This is a very good study that supports a potential role for anti-ribosomal P antibodies in lupus psychosis. Findings strengthened previous reports in the literature about this association. The topic is up-to-date and the results are of high interest for other researchers.

REFERENCES

1 **Karassa FB**, Afeltra A, Ambrozic A, Chang DM, De Keyser F, Doria A, Galeazzi M, Hirohata S, Hoffman IE, Inanc M, Massardo L, Mathieu A, Mok CC, Morozzi G, Sanna G, Spindler AJ, Tzioufas AG, Yoshio T, Ioannidis JP. Accuracy of anti-ribosomal P protein antibody testing for the diagnosis of neuropsychiatric systemic lupus erythematosus: an international meta-analysis. *Arthritis Rheum* 2006; **54**: 312-324 [PMID: 16385548 DOI: 10.1002/art.21539]

2 **Meszaros ZS**, Perl A, Faraone SV. Psychiatric symptoms in systemic lupus erythematosus: a systematic review. *J Clin Psychiatry* 2012; **73**: 993-1001 [PMID: 22687742 DOI: 10.4088/JCP.11m07043]

3 The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 1999; **42**: 599-608 [PMID: 10211873]

4 **American Psychiatric Association.** Diagnostic and statistical manual of mental disorders. 5th ed. Arlington: American Psychiatric Publishing, 2013

5 **Mantovani C**, Louzada-Junior P, Nunes EA, de Figueiredo FP, Oliveira GR, Del-Ben CM. Antinuclear antibodies testing as a routine screening for systemic lupus erythematosus in patients presenting first-episode psychosis. *Early Interv Psychiatry* 2012; **6**: 322-325 [PMID: 22260390 DOI: 10.1111/j.1751-7893.2011.00339.x]

6 **Arnett FC**, Reveille JD, Moutsopoulos HM, Georgescu L, Elkon KB. Ribosomal P autoantibodies in systemic lupus erythematosus. Frequencies in different ethnic groups and clinical and immunogenetic associations. *Arthritis Rheum* 1996; **39**: 1833-1839 [PMID: 8912505 DOI: 10.1002/art.1780391109]

7 **Hanly JG**, Urowitz MB, Siannis F, Farewell V, Gordon C, Bae SC, Isenberg D, Dooley MA, Clarke A, Bernatsky S, Gladman D, Fortin PR, Manzi S, Steinsson K, Bruce IN, Ginzler E, Aranow C, Wallace DJ, Ramsey-Goldman R, van Vollenhoven R, Sturfelt G, Nived O, Sanchez-Guerrero J, Alarcón GS, Petri M, Khamashta M, Zoma A, Font J, Kalunian K, Douglas J, Qi Q, Thompson K, Merrill JT. Autoantibodies and neuropsychiatric events at the time of systemic lupus erythematosus diagnosis: results from an international inception cohort study. *Arthritis Rheum* 2008; **58**: 843-853 [PMID: 18311802 DOI: 10.1002/art.23218]

8 **Hanly JG**, Urowitz MB, Su L, Bae SC, Gordon C, Clarke A, Bernatsky S, Vasudevan A, Isenberg D, Rahman A, Wallace DJ, Fortin PR, Gladman D, Romero-Diaz J, Sanchez-Guerrero J, Dooley MA, Bruce I, Steinsson K, Khamashta M, Manzi S, Ramsey-Goldman R, Sturfelt G, Nived O, van Vollenhoven R, Ramos-Casals M, Aranow C, Mackay M, Kalunian K, Alarcón GS, Fessler BJ, Ruiz-Irastorza G, Petri M, Lim S, Kamen D, Peschken C, Farewell V, Thompson K, Theriault C, Merrill JT. Autoantibodies as biomarkers for the prediction of neuropsychiatric events in systemic lupus erythematosus. *Ann Rheum Dis* 2011; **70**: 1726-1732 [PMID: 21893582 DOI: 10.1136/ard.2010.148502]

9 **Isshi K**, Hirohata S. Association of anti-ribosomal P protein antibodies with neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum* 1996; **39**: 1483-1490 [PMID: 8814059 DOI: 10.1002/art.1780390907]

10 **Nojima Y**, Minota S, Yamada A, Takaku F, Aotsuka S, Yokohari R. Correlation of antibodies to ribosomal P protein with psychosis in patients with systemic lupus erythematosus. *Ann Rheum Dis* 1992; **51**: 1053-1055 [PMID: 1417136 DOI: 10.1136/ard.51.9.1053]

11 **Schneebaum AB**, Singleton JD, West SG, Blodgett JK, Allen LG, Cheronis JC, Kotzin BL. Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am J Med* 1991; **90**: 54-62 [PMID: 1986591 DOI: 10.1016/0002-9343(91)90506-S]

12 **Briani C**, Lucchetta M, Ghirardello A, Toffanin E, Zampieri S, Ruggero S, Scarlato M, Quattrini A, Bassi N, Ermani M, Battistin L, Doria A. Neurolupus is associated with anti-ribosomal P protein antibodies: an inception cohort study. *J Autoimmun* 2009; **32**: 79-84 [PMID: 19171463 DOI: 10.1016/j.jaut.2008.12.002]

13 **Abdel-Nasser AM**, Ghaleb RM, Mahmoud JA, Khairy W, Mahmoud RM. Association of anti-ribosomal P protein antibodies with neuropsychiatric and other manifestations of systemic lupus erythematosus. *Clin Rheumatol* 2008; **27**: 1377-1385 [PMID: 18481154 DOI: 10.1007/s10067-008-0921-1]

14 **Almeida D**, Antolín J, Amérigo MJ, Cantabrana A, Roces A, Hayeck M. [Anti-ribosomal antibodies as activity markers in systemic lupus erythematosus]. *An Med Interna* 2002; **19**: 73-75 [PMID: 11989101]

15 **Bonfa E**, Golombek SJ, Kaufman LD, Skelly S, Weissbach H, Brot N, Elkon KB. Association between lupus psychosis and anti-ribosomal P protein antibodies. *N Engl J Med* 1987; **317**: 265-271 [PMID: 3496538 DOI: 10.1056/NEJM198707303170503]

16 **Caponi L**, Chimenti D, Pratesi F, Migliorini P. Anti-ribosomal antibodies from lupus patients bind DNA. *Clin Exp Immunol* 2002; **130**: 541-547 [PMID: 12452847 DOI: 10.1046/j.1365-2249.2002.02014.x]

17 **Chan EY**, Ko OK, Lawton JW, Lau CS. The use of anti-ribosomal P antibodies in the diagnosis of cerebral lupus--superiority of western blotting over enzyme-linked immunosorbent assay. *Hong Kong Med J* 1998; **4**: 145-150 [PMID: 11832566]

18 **Conti F**, Alessandri C, Bompane D, Bombardieri M, Spinelli FR, Rusconi AC, Valesini G. Autoantibody profile in systemic lupus erythematosus with psychiatric manifestations: a role for anti-endothelial-cell antibodies. *Arthritis Res Ther* 2004; **6**: R366-R372 [PMID: 15225372 DOI: 10.1186/ar1198]

19 **Derksen RH**, van Dam AP, Gmelig Meyling FH, Bijlsma JW, Smeenk RJ. A prospective study on antiribosomal P proteins in two cases of familial lupus and recurrent psychosis. *Ann Rheum Dis* 1990; **49**: 779-782 [PMID: 2241267 DOI: 10.1136/ard.49.10.779]

20 **Eber T**, Chapman J, Shoenfeld Y. Anti-ribosomal P-protein and its role in psychiatric manifestations of systemic lupus erythematosus: myth or reality? *Lupus* 2005; **14**: 571-575 [PMID: 16175927 DOI: 10.1191/0961203305lu2150rr]

21 **Ghirardello A**, Doria A, Zampieri S, Gambari PF, Todesco S. Autoantibodies to ribosomal P proteins in systemic lupus erythematosus. *Isr Med Assoc J* 2001; **3**: 854-857 [PMID: 11729585]

22 **Haddouk S**, Marzouk S, Jallouli M, Fourati H, Frigui M, Hmida YB, Koubaa F, Sellami W, Baklouti S, Hachicha J, Bahloul Z, Masmoudi H. Clinical and diagnostic value of ribosomal P autoantibodies in systemic lupus erythematosus. *Rheumatology* (Oxford) 2009; **48**: 953-957 [PMID: 19502474 DOI: 10.1093/rheumatology/kep142]

23 **Hoffman IE**, Peene I, Meheus L, Huizinga TW, Cebecauer L, Isenberg D, De Bosschere K, Hulstaert F, Veys EM, De Keyser F. Specific antinuclear antibodies are associated with clinical features in systemic lupus erythematosus. *Ann Rheum Dis* 2004; **63**: 1155-1158 [PMID: 15308527 DOI: 10.1136/ard.2003.013417]

24 **Isshi K**, Hirohata S. Differential roles of the anti-ribosomal P antibody and antineuronal antibody in the pathogenesis of central nervous system involvement in systemic lupus erythematosus. *Arthritis Rheum* 1998; **41**: 1819-1827 [PMID: 9778223]

25 **Jönsen A**, Bengtsson AA, Nived O, Ryberg B, Truedsson L, Rönnblom L, Alm GV, Sturfelt G. The heterogeneity of neuropsychiatric systemic lupus erythematosus is reflected in lack of association with cerebrospinal fluid cytokine profiles. *Lupus* 2003; **12**: 846-850 [PMID: 14667101 DOI: 10.1191/0961203303lu472sr]

26 **Kao CH**, Ho YJ, Lan JL, Changlai SP, Liao KK, Chieng PU. Discrepancy between regional cerebral blood flow and glucose metabolism of the brain in systemic lupus erythematosus patients with normal brain magnetic resonance imaging findings. *Arthritis Rheum* 1999; **42**: 61-68 [PMID: 9920015]

27 **Magalhães MB**, da Silva LM, Voltarelli JC, Donadi EA, Louzada-Junior P. Lymphocytotoxic antibodies in systemic lupus erythematosus are associated with disease activity irrespective of the presence of neuropsychiatric manifestations. *Scand J Rheumatol* 2007; **36**: 442-447 [PMID: 18092265 DOI: 10.1080/03009740701482768]

28 **Mahler M**, Ngo JT, Schulte-Pelkum J, Luettich T, Fritzler MJ. Limited reliability of the indirect immunofluorescence technique for the detection of anti-Rib-P antibodies. *Arthritis Res Ther* 2008; **10**: R131 [PMID: 19000323 DOI: 10.1186/ar2548]

29 **Massardo L**, Burgos P, Martínez ME, Pérez R, Calvo M, Barros J, González A, Jacobelli S. Antiribosomal P protein antibodies in Chilean SLE patients: no association with renal disease. *Lupus* 2002; **11**: 379-383 [PMID: 12139376 DOI:

10.1191/0961203302lu209oa]
30 **Muñoz-Málaga A**, Anglada JC, Páez M, Girón JM, Barrera A. [Psychosis as the initial manifestation of systemic lupus erythematosus: the role of lupus band test and anti-ribosomal antibodies]. *Rev Neurol* 1999; **28**: 779-781 [PMID: 10363321]
31 **Nagai T**, Arinuma Y, Yanagida T, Yamamoto K, Hirohata S. Anti-ribosomal P protein antibody in human systemic lupus erythematosus up-regulates the expression of proinflammatory cytokines by human peripheral blood monocytes. *Arthritis Rheum* 2005; **52**: 847-855 [PMID: 15751081 DOI: 10.1002/art.20869]
32 **Nagai T**, Yanagida T, Hirohata S. Anti-ribosomal P protein antibody induces Th1 responses by enhancing the production of IL-12 in activated monocytes. *Mod Rheumatol* 2011; **21**: 57-62 [PMID: 20824297 DOI: 10.1007/s10165-010-0354-y]
33 **Press J**, Palayew K, Laxer RM, Elkon K, Eddy A, Rakoff D, Silverman ED. Antiribosomal P antibodies in pediatric patients with systemic lupus erythematosus and psychosis. *Arthritis Rheum* 1996; **39**: 671-676 [PMID: 8630119 DOI: 10.1002/art.1780390420]
34 **Sanna G**, Piga M, Terryberry JW, Peltz MT, Giagheddu S, Satta L, Ahmed A, Cauli A, Montaldo C, Passiu G, Peter JB, Shoenfeld Y, Mathieu A. Central nervous system involvement in systemic lupus erythematosus: cerebral imaging and serological profile in patients with and without overt neuropsychiatric manifestations. *Lupus* 2000; **9**: 573-583 [PMID: 11035431 DOI: 10.1191/096120300678828695]
35 **Sato T**, Uchiumi T, Ozawa T, Kikuchi M, Nakano M, Kominami R, Arakawa M. Autoantibodies against ribosomal proteins found with high frequency in patients with systemic lupus erythematosus with active disease. *J Rheumatol* 1991; **18**: 1681-1684 [PMID: 1787488]
36 **Shovman O**, Zandman-Goddard G, Gilburd B, Blank M, Ehrenfeld M, Bardechevski S, Stojanovich L, Langevitz P, Shoenfeld Y. Restricted specificity of anti-ribosomal P antibodies to SLE patients in Israel. *Clin Exp Rheumatol* 2006; **24**: 694-697 [PMID: 17207387]
37 **Teh LS**. Antiribosomal P antibodies and lupus psychosis. *Ann Rheum Dis* 1992; **51**: 1104 [PMID: 1417156]
38 **Teh LS**, Hay EM, Amos N, Black D, Huddy A, Creed F, Bernstein RM, Holt PJ, Williams BD. Anti-P antibodies are associated with psychiatric and focal cerebral disorders in patients with systemic lupus erythematosus. *Br J Rheumatol* 1993; **32**: 287-290 [PMID: 8461921 DOI: 10.1093/rheumatology/32.4.287]
39 **Teh LS**, Lee MK, Wang F, Manivasagar M, Charles PJ, Nicholson GD, Hay EM, Isenberg DA, Amos N, Williams BD. Antiribosomal P protein antibodies in different populations of patients with systemic lupus erythematosus. *Br J Rheumatol* 1993; **32**: 663-665 [PMID: 8348266 DOI: 10.1093/rheumatology/32.8.663]
40 **Toubi E**, Shoenfeld Y. Clinical and biological aspects of anti-P-ribosomal protein autoantibodies. *Autoimmun Rev* 2007; **6**: 119-125 [PMID: 17289545 DOI: 10.1016/j.autrev.2006.07.004]
41 **Tzioufas AG**, Tzortzakis NG, Panou-Pomonis E, Boki KA, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM. The clinical relevance of antibodies to ribosomal-P common epitope in two targeted systemic lupus erythematosus populations: a large cohort of consecutive patients and patients with active central nervous system disease. *Ann Rheum Dis* 2000; **59**: 99-104 [PMID: 10666163 DOI: 10.1136/ard.59.2.99]
42 **van Dam AP**. Diagnosis and pathogenesis of CNS lupus. *Rheumatol Int* 1991; **11**: 1-11 [PMID: 1866569 DOI: 10.1007/BF00290244]
43 **Watanabe T**, Sato T, Uchiumi T, Arakawa M. Neuropsychiatric manifestations in patients with systemic lupus erythematosus: diagnostic and predictive value of longitudinal examination of anti-ribosomal P antibody. *Lupus* 1996; **5**: 178-183 [PMID: 8803887 DOI: 10.1177/096120339600500303]
44 **Weiner SM**, Otte A, Schumacher M, Klein R, Gutfleisch J, Brink I, Otto P, Nitzsche EU, Moser E, Peter HH. Diagnosis and monitoring of central nervous system involvement in systemic lupus erythematosus: value of F-18 fluorodeoxyglucose PET. *Ann Rheum Dis* 2000; **59**: 377-385 [PMID: 10784521 DOI: 10.1136/ard.59.5.377]
45 **West SG**, Emlen W, Wener MH, Kotzin BL. Neuropsychiatric lupus erythematosus: a 10-year prospective study on the value of diagnostic tests. *Am J Med* 1995; **99**: 153-163 [PMID: 7625420 DOI: 10.1016/S0002-9343(99)80135-1]
46 **Williams RC**, Sugiura K, Tan EM. Antibodies to microtubule-associated protein 2 in patients with neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum* 2004; **50**: 1239-1247 [PMID: 15077307 DOI: 10.1002/art.20156]
47 **Yalaoui S**, Gorgi Y, Hajri R, Goucha R, Chaabouni L, Kooli C, Ayed K. Autoantibodies to ribosomal P proteins in systemic lupus erythematosus. *Joint Bone Spine* 2002; **69**: 173-176 [PMID: 12027308 DOI: 10.1016/S1297-319X(02)00366-4]
48 **Yoshio T**, Masuyama J, Ikeda M, Tamai K, Hachiya T, Emori T, Mimori A, Takeda A, Minota S, Kano S. Quantification of antiribosomal P0 protein antibodies by ELISA with recombinant P0 fusion protein and their association with central nervous system disease in systemic lupus erythematosus. *J Rheumatol* 1995; **22**: 1681-1687 [PMID: 8523345]
49 **Hunter J,** Schmidt F. Fixed Effects vs. Random Effects Meta-Analysis Models: Implications for Cumulative Research Knowledge. *Inter J Select Assess* 2000; **8**: 275-292 [DOI: 10.1111/1468-2389.00156]
50 **Cochran WG**. The comparison of percentages in matched samples. *Biometrika* 1950; **37**: 256-266 [PMID: 14801052]
51 **Song F**, Sheldon TA, Sutton AJ, Abrams KR, Jones DR. Methods for exploring heterogeneity in meta-analysis. *Eval Health Prof* 2001; **24**: 126-151 [PMID: 11523383]
52 **Greenland S**, Robins JM. Conceptual problems in the definition and interpretation of attributable fractions. *Am J Epidemiol* 1988; **128**: 1185-1197 [PMID: 3057878]
53 **Zalcman S**, Green-Johnson JM, Murray L, Nance DM, Dyck D, Anisman H, Greenberg AH. Cytokine-specific central monoamine alterations induced by interleukin-1, -2 and -6. *Brain Res* 1994; **643**: 40-49 [PMID: 7518332 DOI: 10.1016/0006-8993(94)90006-X]
54 **Gaspar PA**, Bustamante ML, Silva H, Aboitiz F. Molecular mechanisms underlying glutamatergic dysfunction in schizophrenia: therapeutic implications. *J Neurochem* 2009; **111**: 891-900 [PMID: 19686383 DOI: 10.1111/j.1471-4159.2009.06325.x]
55 **Hirohata S**, Kanai Y, Mitsuo A, Tokano Y, Hashimoto H. Accuracy of cerebrospinal fluid IL-6 testing for diagnosis of lupus psychosis. A multicenter retrospective study. *Clin Rheumatol* 2009; **28**: 1319-1323 [PMID: 19593599 DOI: 10.1007/s10067-009-1226-8]
56 **Ezeoke A**, Mellor A, Buckley P, Miller B. A systematic, quantitative review of blood autoantibodies in schizophrenia. *Schizophr Res* 2013; **150**: 245-251 [PMID: 23953827 DOI: 10.1016/j.schres.2013.07.029]
57 **Gilat Y,** Shoenfeld Y, Kotler M, Iancu I. Anti-ribosomal P antibody in schizophrenia. *Isr J Psychiatry Relat Sci* 2011; **48**: 275-279 [PMID: 22572091]
58 **Chen SJ**, Chao YL, Chen CY, Chang CM, Wu EC, Wu CS, Yeh HH, Chen CH, Tsai HJ. Prevalence of autoimmune diseases in in-patients with schizophrenia: nationwide population-based study. *Br J Psychiatry* 2012; **200**: 374-380 [PMID: 22442099 DOI: 10.1192/bjp.bp.111.092098]
59 **Eaton WW**, Byrne M, Ewald H, Mors O, Chen CY, Agerbo E, Mortensen PB. Association of schizophrenia and autoimmune diseases: linkage of Danish national registers. *Am J Psychiatry* 2006; **163**: 521-528 [PMID: 16513876 DOI: 10.1176/appi.ajp.163.3.521]
60 **Eaton WW**, Pedersen MG, Nielsen PR, Mortensen PB. Autoimmune diseases, bipolar disorder, and non-affective psychosis. *Bipolar Disord* 2010; **12**: 638-646 [PMID: 20868462 DOI:

10.1111/j.1399-5618.2010.00853.x]
61 **Purcell SM**, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 2009; **460**: 748-752 [PMID: 19571811 DOI: 10.1038/nature08185]
62 **Shi J**, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I, Dudbridge F, Holmans PA, Whittemore AS, Mowry BJ, Olincy A, Amin F, Cloninger CR, Silverman JM, Buccola NG, Byerley WF, Black DW, Crowe RR, Oksenberg JR, Mirel DB, Kendler KS, Freedman R, Gejman PV. Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature* 2009; **460**: 753-757 [PMID: 19571809 DOI: 10.1038/nature08192]
63 **Stefansson H**, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, Werge T, Pietiläinen OP, Mors O, Mortensen PB, Sigurdsson E, Gustafsson O, Nyegaard M, Tuulio-Henriksson A, Ingason A, Hansen T, Suvisaari J, Lonnqvist J, Paunio T, Børglum AD, Hartmann A, Fink-Jensen A, Nordentoft M, Hougaard D, Norgaard-Pedersen B, Böttcher Y, Olesen J, Breuer R, Möller HJ, Giegling I, Rasmussen HB, Timm S, Mattheisen M, Bitter I, Réthelyi JM, Magnusdottir BB, Sigmundsson T, Olason P, Masson G, Gulcher JR, Haraldsson M, Fossdal R, Thorgeirsson TE, Thorsteinsdottir U, Ruggeri M, Tosato S, Franke B, Strengman E, Kiemeney LA, Melle I, Djurovic S, Abramova L, Kaleda V, Sanjuan J, de Frutos R, Bramon E, Vassos E, Fraser G, Ettinger U, Picchioni M, Walker N, Toulopoulou T, Need AC, Ge D, Yoon JL, Shianna KV, Freimer NB, Cantor RM, Murray R, Kong A, Golimbet V, Carracedo A, Arango C, Costas J, Jönsson EG, Terenius L, Agartz I, Petursson H, Nöthen MM, Rietschel M, Matthews PM, Muglia P, Peltonen L, St Clair D, Goldstein DB, Stefansson K, Collier DA. Common variants conferring risk of schizophrenia. *Nature* 2009; **460**: 744-747 [PMID: 19571808 DOI: 10.1038/nature08186]
64 **McAllister CG**, Rapaport MH, Pickar D, Podruchny TA, Christison G, Alphs LD, Paul SM. Increased numbers of CD5+ B lymphocytes in schizophrenic patients. *Arch Gen Psychiatry* 1989; **46**: 890-894 [PMID: 2478093 DOI: 10.1001/archpsyc.1989.01810100032006]
65 **Printz DJ**, Strauss DH, Goetz R, Sadiq S, Malaspina D, Krolewski J, Gorman JM. Elevation of CD5+ B lymphocytes in schizophrenia. *Biol Psychiatry* 1999; **46**: 110-118 [PMID: 10394480 DOI: 10.1016/S0006-3223(98)00307-2]
66 **Steiner J**, Jacobs R, Panteli B, Brauner M, Schiltz K, Bahn S, Herberth M, Westphal S, Gos T, Walter M, Bernstein HG, Myint AM, Bogerts B. Acute schizophrenia is accompanied by reduced T cell and increased B cell immunity. *Eur Arch Psychiatry Clin Neurosci* 2010; **260**: 509-518 [PMID: 20107825 DOI: 10.1007/s00406-010-0098-x]

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META-ANALYSIS

Statin use and risk of liver cancer: A meta-analysis of 7 studies involving more than 4.7 million patients

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Abstract

AIM: To pool data currently available to determine the association between statin use and the risk of liver cancer.

METHODS: A computerized literature search was conducted to identify those relevant studies between January 1966 and March 2013. Stata 11.0 (Stata Corp, College Station, Texas) was used for statistical analyses. Pooled relative risk (RR) estimates with 95%CI were calculated for overall analysis and subgroup analyses, using the random- and fixed-effects models. Heterogeneities between studies were evaluated by Cochran's *Q* test and I^2 statistic. The Begg's funnel plot and Egger's regression asymmetry test were used to detect the publication bias.

RESULTS: Seven studies were included in our meta-analysis according to the selection criteria, including four cohort studies and three case-control studies. These studies involved 4725593 people and 9785 liver cancer cases. The overall analysis showed that statin use was statistically associated with a significantly reduced risk of liver cancer (random-effects model, RR = 0.61, 95%CI: 0.49-0.76, $P < 0.001$; fixed-effects model, RR = 0.64, 95%CI: 0.57-0.71, $P < 0.001$); however, significant heterogeneity was found between studies (Cochran's Q statistic = 19.13, $P = 0.004$; $I^2 = 68.6\%$). All subgroup analyses provided supporting evidence for the results of overall analysis. Begg's ($Z = 0.15$, $P = 0.881$) and Egger's test ($t = -0.44$, $P = 0.681$) showed no significant risk of having a publication bias.

CONCLUSION: Statin use was associated with the reduced risk of liver cancer. To clearly clarify this relationship, more high quality studies are required.

Key words: Statin use; Liver cancer; Reduced risk; Meta-analysis

Core tip: Statin use has been suggested to be associated with the risk of liver cancer by some studies, but no consensus was reached among them. This meta-analysis involved 4725593 people, 9785 liver cancer cases, and found that statin use was associated with the reduced risk of liver cancer (RR = 0.67, 95%CI: 0.55-0.82, $P < 0.001$).

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INTRODUCTION

Liver cancer, with a mounting annual incidence of 4.9 per 100000 people, is the third most common cause of cancer death worldwide. For example in 2008, an estimated 748300 new liver cancer cases and 695900 cancer deaths occurred[1]. Despite some advances in treatment over the past several decades, the prognosis of liver cancer is unfavorable. Even in the most developed countries like the United States, the 1-year survival rate is less than 50%[2]. Because of its high fatality rate, it is very important to identify those risk and protective factors. Major risk factors that were identified include hepatitis B virus (HBV), hepatitis C virus (HCV), cirrhosis, heavy alcoholic consumption, non-alcoholic steatohepatitis, and alfatoxin exposure[3]. Recently, emerging evidence suggests that diabetes mellitus (DM) may be a potential risk factor for liver cancer[4,5], whereas metformin use in diabetic patients, coffee and tea consumption have been suggested as possible protective factors[6-8].

Statins have been widely used to lower the cholesterol level, which could inhibit the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-COA), the rate-limiting enzyme in the mevalonate synthesis pathway[9]. The protective role of statin use in cardiovascular diseases has been confirmed by several large-scale randomized controlled trials (RCTs)[10-13]. Over the past several years, statins have been suggested to be associated with some varieties of cancers, including liver cancer[14-16]. For example, two epidemiological studies which were performed in Taiwan province of China found that statin use was associated with the reduced risk of liver cancer[17,18]. In addition, *in vitro* and *in vivo* studies showed that statins could enhance anti-proliferative effects of some antitumor agents in cancer treatment[19-21]. Moreover, combined treatment with pravastatin and chemoembolization had been reported to improve the survival rate of patients with hepatocellular carcinoma (HCC)[22].

However, results from the limited number of RCTs were disappointing[23], which could not provide supportive evidence for those above-mentioned epidemiological and pre-clinical studies, although several limitations should be acknowledged in these RCTs. First, most of these studies were not designed to determine the association of statin use with the risk of liver cancer. Therefore, liver cancer was neither a primary end point nor a topic of interest in these studies, and selection bias may not be avoided[24]. The second limitation is that most of these studies were conducted in the United States or European countries, not in the areas with higher incidence of HCC, such as China and other Asian countries. Thus, the number of observed liver cancer cases was very limited[25], which could not be regarded as the representative for the overall study population. Considering that no consensus was reached among these studies, this meta-analysis was performed to pool data currently available to determine the association between statin use and the risk of liver cancer.

MATERIALS AND METHODS

Search strategy

A computerized literature search (Medline, Embase and the Cochrane library) was conducted to identify those relevant studies between January 1966 and March 2013. The Medical subject heading (MeSH) terms and/or the text words which were used included "statin(s)", "HMG-COA reductase inhibitor(s)", "atorvastatin", "cerivastatin", "fluvastatin", "lovastatin", "mevastatin", "pravastatin", "rivastatin", "rosuvastatin", or "simvastatin", combined with "carcinoma(s)", "hepatoma(s)", "cancer(s)", "neoplasm(s)", or "malignancy(ies)". Publication type was limited to "article". Data from abstracts, review articles, editorials, case reports, and letters were excluded. After scanning of the titles and abstracts, studies identified in the search clearly not relevant to our topic of interest were excluded. The full texts of the remaining studies were read to determine whether they were eligible for inclusion. Data from each eligible study were extracted. According to the retrieved original studies and relevant review articles, we also manually searched the reference lists to identify those possible eligible articles which were not found in our primary search.

Selection criteria

The studies would be included in our statistical analysis if they fulfilled these criteria as follows: (1) Epidemiologic studies on human subjects, including cohort study or case-control study; (2) The language was English and only full papers were included; (3) They were designed to evaluate the association between statin use and the risk of liver cancer; and (4) Risk estimates, including relative risks (RRs) for cohort studies or odds ratios (ORs) for case-control studies, and their corresponding 95% confidence intervals (95%CIs) were provided or could be calculated based on the available data. If more than one paper was derived from the same research, only the recently published paper which provided the most abundant information was included. When necessary, the authors were contacted to obtain the corresponding required information.

Data extraction

Each potentially eligible study was evaluated independently by at least two authors (HZ, CG and LF). If evaluation results diverged, agreement was reached in a joint session. The following information would be extracted and recorded, including the first author, the year of publication, study design, country or area which the study was conducted, study time or mean follow-up years, total participants, liver cancer cases, adjusted RR or OR with their 95%CIs, and the confounding factors which had been adjusted. The quality of included studies was not tried to be assessed, considering that no consensus standardized method could be obtained for the quality assessment of observational studies[26,27]. Instead, we performed several subgroup analyses to explore the source of heterogeneity and validate the results from overall analysis.

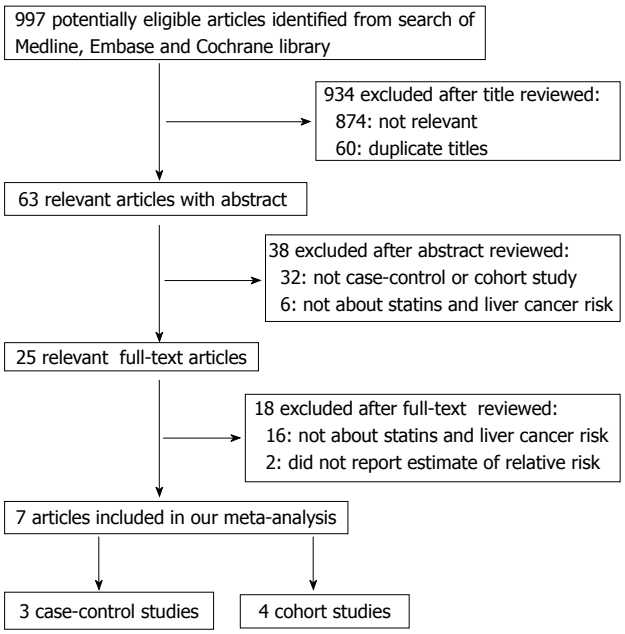

Figure 1 Flow chart of the selection of studies for inclusion.

Statistical analysis

For cohort studies, the value of RRs were used to assess the risk estimate; however, for case-control studies, those ORs would be regarded as approximate RRs in our meta-analysis, considering that the prevalence of liver cancer was relatively very low in these studies. We used the adjusted RRs (ARRs) to estimate the risk of liver cancer treated with statins, whereas for those studies in which no ARR was available, the unadjusted RRs were adopted. We also expressed the summary of results as the RR and the corresponding 95%CI. For all tests, $P < 0.05$ was considered statistically significant unless specially described and all P values quoted were two-sided.

For overall analysis, both random-effects model (DerSimonian and Laird method) and fixed-effects model (Mantel-Haenszel method) were used to calculate the pooled RR estimates[28,29], in order to provide accurate results and conclusions[30,31]. When significant heterogeneity between studies is not found, the two models would provide the similar results, whereas if significant heterogeneity is found, the random-effects model, which incorporates an estimate of between-study variance (heterogeneity) in the weighting, is more appropriate. The combined RR was displayed in Forest plot.

Moreover, subgroup analyses would be performed based on the study design, the country or area in which the study was conducted, the study population and whether the confounding factors had been controlled adequately, in order: (1) to validate the results and conclusion from the overall analysis in different conditions; (2) to further explore the stability and reliability of the overall analysis; and (3) to find the possible source of statistical heterogeneity among studies.

For the statistical heterogeneity among studies, Cochrane Q statistic test with a significance level of $P < 0.10$ was used[32]. I^2 statistic, which describes the percentage variation across studies that is due to heterogeneity rather than chance, was also calculated, considering the low power of Cochrane Q test when the number of included studies was very limited. The heterogeneity would be considered significant when $I^2 > 50\%$[33]. Finally, the publication bias for included studies would be detected using the Begg's funnel plot and Egger's regression asymmetry test[34,35]. We followed the guidelines for the meta-analysis of observational studies in epidemiology proposed by MOOSE group[36]. Stata 11.0 (Stata Corp, College Station, Texas) was used for the statistical analyses.

RESULTS

Search results

Seven studies were included in our meta-analysis according to the selection criteria using the defined MeSH terms and/or the text words, including 4 cohort studies and 3 case-control studies (Table 1 and Figure 1)[17,18,37-41]. Among them, four studies were conducted in European and American regions (United States and Denmark)[37,39-41], and others were in Asian country (Taiwan of China)[17-18,38].

Baseline characteristics of included studies

These studies involved 4725593 people and 9785 liver cancer cases. They were published between the years of 1966 and 2013. The confounding factors which had been controlled in these studies include age, sex, HBV infection, HCV infection, alcohol liver disease, DM, liver cirrhosis, other lipid-lowering drugs, nonsteroidal anti-inflammatory drugs/aspirin, angiotensin-converting enzyme inhibitors, the number of hospitalization, anti-HBV treatment, income, level of urbanization, calendar period, hormone replacement therapy, race, anti-HCV treatment, propensity to use statins, body mass index, and smoking[17,18,37-41]. For these factors, some were controlled by matching which had been indicated in Table 1, and others were controlled by multivariate analyses. When these 5 factors had been controlled, including age, sex, HBV infection, HCV infection and alcohol liver disease, the confounding factors would be regarded as been controlled adequately.

For the study population, most of the studies (5/7) were designed to aim at the general population, except for two studies: one case-control study limited their patients to those with DM[37], and another cohort study restricted to those with HBV infection[18]. In the study performed by Friedman *et al*[40] in the United States, 4222660 patients were observed and only 32 patients were diagnosed with liver cancer, including intrahepatic bile duct cancer cases. In addition, in this study[40] the RRs and their 95%CIs were reported by men and women, separately. Therefore, we had pooled the two risk estimates before statistical analysis, using random-effects model and fixed-effects model; and the two models yielded a same result (Table 1).

Table 1 Baseline characteristics and results of multivariate analysis in included studies

Ref.	Country	Study time/mean follow-up years	Total participants	Liver cancer cases	Adjusted RR(95%CI)	Confounding adjustment[1]
Case-control studies (n = 3)						
El-Serag *et al*[37]	United State	1997-2002	6515	1303	0.74 (0.64-0.87)	1[2], 2[2], 3-5, 6[2], 7, 9, 10, 17-19
Chiu *et al*[17]	Taiwan	2005-2008	2332	312	0.62 (0.45-0.83)	1, 2, 3[2], 4[2], 5-10
Leung et al[38]	Taiwan	2000-2008	34205	6841	0.44 (0.28-0.72)	1[2], 2[2], 6, 8, 9,
Cohort studies (n = 4)						
Friis *et al*[39]	Denmark	3.3 (exposure) 5.1 (control)	334754	171	1.16 (0.46-2.90)	1, 2, 9, 15, 16
Friedman *et al*[40]	United State	4.91	4222660	32	0.47 (0.34-0.64)	15
Marelli *et al*[41]	United State	4.7 (exposure) 4.6 (control)	91714	105	0.88 (0.60-1.28)	1[2], 2[2], 15[2], 17[2], 20[2], 21[2]
Tsan *et al*[18]	Taiwan	328196 (person-years)	33413	1021	0.47 (0.36-0.61)	1-14

[1]1: Age; 2: Sex; 3: HBV infection; 4: HCV infection; 5: Alcohol liver disease; 6: Diabetes mellitus; 7: Liver cirrhosis; 8: Other lipid-lowering drugs; 9: Nonsteroidal anti-inflammatory drugs/aspirin; 10: Angiotensin-converting enzyme inhibitors; 11: Number of hospitalization; 12: anti-HBV treatment; 13: Income; 14: Level of urbanization; 15: Calendar period; 16: Hormone replacement therapy; 17: Race; 18: Anti-HCV treatment; 19: Propensity to use statins; 20: Body mass index; 21: Smoking. [2]Variables which had been indicated were controlled by matching, and others were controlled by multivariate analyses. RR: Relative risk; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

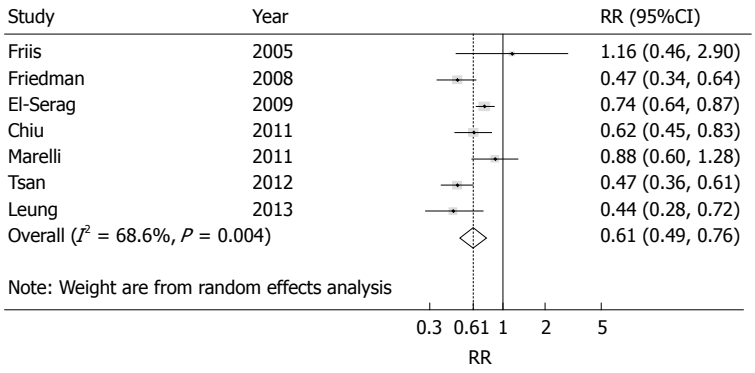

Figure 2 Forest plot of pooled relative risks and their 95%CI for statin use and the risk of liver cancer, when using random effects model. Studies are arranged based on the year of publication. Black boxes indicate the relative risks point estimate, and their areas are proportional to the weights of the studies. Horizontal lines represent the 95%CIs. The broken line and diamond represent the summary estimate and the unbroken vertical line is at the null value.

Overall analysis: Reduced risk of liver cancer with statin use

Table 1 shows the adjusted RRs, their corresponding 95%CIs, and the confounding adjustment in included studies. The RRs and their 95%CIs from the study by Friedman *et al*[40] had been pre-treated before the final statistical analysis. Of the total seven studies, reduced risk of liver cancer was observed in five studies by multivariate analysis, whereas the results with no statistical difference had been demonstrated in the left two studies. For all the three case-control studies, positive results were found; however, for the four cohort studies, half of them demonstrated no statistical difference. We used the random- and fixed-effects models to perform the overall analysis (Figure 2). The results showed that statin use was statistically significantly associated with the reduced risk of liver cancer (random-effects model, RR = 0.61, 95%CI: 0.49-0.76, $P < 0.001$; fixed-effects model, RR = 0.64, 95%CI: 0.57-0.71, $P < 0.001$); however, significant heterogeneity was found between studies (Cochran's Q statistic = 19.13, $P = 0.004$, $I^2 = 68.6\%$, Figure 2).

Subgroup analyses

We further performed subgroup analyses to validate the results from the overall analysis, and to find the possible source of statistical heterogeneity among studies. As shown in Table 2, subgroup analyses were performed according to the type of design of studies, the country or area in which the study was conducted, the study population and whether the confounding factors had been controlled adequately. Based on the results of Cochran's Q statistic, significant heterogeneities were not found only when the subgroup analysis was restricted into those studies which were conducted in Asian country. This may be because these three studies were conducted in the same area, Taiwan province of China (Table 2).

Fortunately, all of the subgroup analyses provided supporting evidence for the results of overall analysis, especially when the subgroup analysis was restricted into those cohort studies. The results from the four cohort studies also showed that statin use was associated with the reduced risk of liver cancer (random-effects model, RR = 0.62, 95%CI: 0.43-0.89, $P = 0.010$; fixed-effects model, RR = 0.56, 95%CI: 0.47-0.66, $P < 0.001$), although heterogeneity was also found (Cochran's Q = 10.74, $P = 0.013$, $I^2 = 72.1\%$).

Publication bias

Finally, we detected the publication bias using the Begg's funnel plot and Egger's regression asymmetry test. As shown

Table 2 Subgroup analyses of included studies

Subgroup	No. of studies	Fixed-effects model			Random-effects model			Heterogeneity		
		RR	95%CI	*P* value	RR	95%CI	*P* value	*Q*	*P*	I^2 (%)
Type of design of studies										
Case-control	3	0.69	0.60-0.79	< 0.001	0.63	0.49-0.82	< 0.001	4.75	0.093	57.9
Cohort	4	0.56	0.47-0.66	< 0.001	0.62	0.43-0.89	0.01	10.74	0.013	72.1
Country or area										
Asian	3	0.51	0.43-0.62	< 0.001	0.51	0.42-0.63	< 0.001	2.30	0.317	13.0
Euro- American	4	0.71	0.62-0.80	< 0.001	0.71	0.52-0.95	0.023	9.14	0.028	67.2
Confounding adjustment										
Adequately[1]	3	0.65	0.58-0.74	< 0.001	0.61	0.46-0.81	0.001	8.64	0.013	76.8
Inadequately	4	0.59	0.48-0.73	< 0.001	0.63	0.41-0.95	0.028	9.82	0.020	69.4
Study population										
General population	5	0.60	0.50-0.71	< 0.001	0.62	0.46-0.83	0.001	9.89	0.042	59.5
Restricted to specified patients[2]	2	0.66	0.58-0.75	< 0.001	0.60	0.38-0.93	0.023	8.50	0.004	88.2

[1]The confounding factors would be regarded as been controlled adequately when these 5 factors had been controlled, including age, sex, HBV infection, HCV infection and alcohol liver disease; [2]The study population of two studies was restricted to specified patients, including patients with diabetes mellitus (Ref. 37) and those with HBV infection (Ref. 18). RR: Relative risk; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

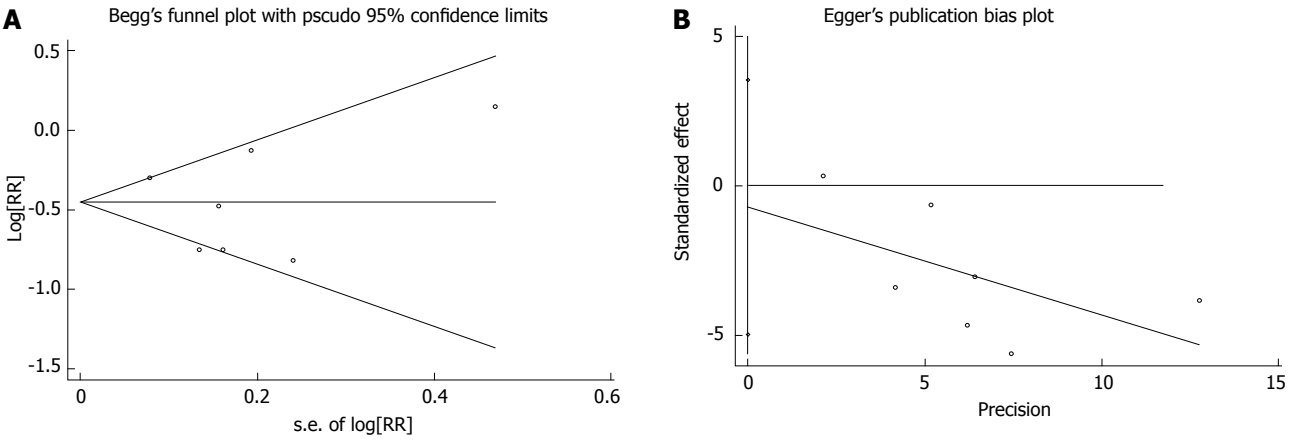

Figure 3 Publication bias detected by the Begg's funnel plot (A) and Egger's regression asymmetry test (B).

in Figure 3 by Begg's (Z = 0.15, P = 0.881) and Egger's test (t = -0.44, P = 0.681), no statistically significant publication bias was noted.

DISCUSSION

Our meta-analysis, which involved 4725593 people and 9785 liver cancer cases, was designed to determine the association between statin use and the risk of liver cancer. The results showed that statin use was associated with a 36%-39% reduction in liver cancer risk. Moreover, all subgroup analyses provided supporting evidence for the results of overall analysis. In addition, no significant risk of having a publication bias was observed by using Begg's plot and Egger's regression test.

Statins have been widely and successfully used in patients with hypercholesterolemia and cardiovascular diseases for more than 30 years[42]. In the past few years, some studies have been designed to determine the association between statins use and the risk of cancer, including liver cancer, taking into account the large number of patient population treated with statins. Besides the epidemiological studies, preclinical studies also suggested that statins may inhibit the growth of cancer cells by promoting apoptosis, suppressing angiogenesis and inhibiting the metastatic process[43-45]. Considering that no consensus was reached among these studies, our meta-analysis was performed to pool data currently available to determine this relationship.

Some issues or questions, which could be regarded as the drawbacks or limitations, should be acknowledged, before acceptance of these results and conclusion. The first was about the observational designs of included studies, which could not provide definite evidence to clarify the causal association. Of the total seven studies, four were population-based cohort designs, three were case-control designs, and none was prospective intervention study. Unfortunately, for the four cohort studies, half of them were shown as without statistical difference. From the viewpoint of basic principle, cohort study is better than case-control study to explain the causal association[24]. However, even when the study population

was limited to those from cohort studies, consistent results were obtained which showed that statin use was associated with the reduced risk of liver cancer, providing supporting evidence for the results of overall analysis. To clarify this relationship between statin use and liver cancer risk, more cohort studies, especially prospective intervention studies, are required.

The second was about the very limited number of included studies. To include all the possibly relevant studies in which the study population could represent the majority of the general population, we added case-control studies in the statistical analysis and treated the ORs as approximate RRs, which may have some effect on the final results. However, when the study population was restricted to those either from cohort studies or from case-control studies, the results remained unchanged. Be that as it may, we also hope that these results and conclusions could be validated in more patients, more hospitals and more countries with higher quality.

The third was about the study population and the general population. The seven studies were conducted in three countries, including the United States, Denmark and Taiwan (China), which could not be regarded as the representative of the general population. Mainland China has the higher incidence of liver cancer with nearly 40 per 100000 people per year, which is more than eight times compared with the average incidence worldwide. In addition, risk factors, including the subtype of HBV, in Taiwan are different from those in mainland China. Therefore, for the general study population, more studies are required, such as prospective intervention study in China.

The fourth limitation was about the confounding factors which could not be controlled adequately because of the original nature of observational epidemiological studies. These studies did not have the process of random allocation, and complete controlling of the confounding factors was seemingly impossible. Some important factors, such as age, sex, HBV/HCV infection, cirrhosis and alcohol drinking, were not controlled adequately in some of these studies. Others included: the exposure time of statins was not long enough, leading to one possibility that the positive association may be affected by other factors, such as high socioeconomic status[46,47]; different units and different kind of statins were used in these studies, whereas different statins may have different effects on liver cancer risk, for example, the effect of hydrophilic statins was different from that of hydrophobic statins[48-50]; and statin use was contraindicated in the presence of liver diseases[40], which may have effects on the results and conclusions.

Besides these aforementioned weaknesses, some strengths were made in this meta- analysis to ensure the accuracy and reliability of our results, based on the available literature and current knowledge. The first was about the subgroup analysis which was designed to validate the results from the overall analysis, and to find the possible source of statistical heterogeneity among studies. Fortunately, all of the subgroup analyses provided supporting evidence for the results of overall analysis, especially when the subgroup analysis was restricted into those cohort studies. The second was that our meta-analysis was designed to pool the data currently available to determine the association between statin use and liver cancer risk. For example, in one study[40] the RRs and their 95%CIs were reported by men and women, separately. We had pooled the two risk estimates before statistical analysis, using random-effects model and fixed-effects model, and the two models yielded a same result.

In conclusion, our meta-analysis showed that statin use was associated with the reduced risk of liver cancer. To clearly clarify this relationship, more high quality epidemiological studies, especially prospective intervention studies, are required. *In vitro* data and animal studies are also required to clarify the relevant mechanisms.

COMMENTS

Background

Statin use has been suggested to be associated with the risk of liver cancer by some studies, but no consensus was reached among them.

Research frontiers

Over the past several years, statins have been suggested to be associated with some varieties of cancers, including liver cancer. For example, two epidemiological studies which were performed in Taiwan province of China found that statin use was associated with the reduced risk of liver cancer. In addition, *in vitro* and *in vivo* studies showed that statins could enhance anti-proliferative effects of some antitumor agents in cancer treatment. However, results from the limited number of randomized controlled trials (RCTs) were disappointing, which could not provide supportive evidence for those above-mentioned epidemiological and pre-clinical studies

Innovations and breakthroughs

This meta-analysis involved seven studies with 4725593 people and 9785 liver cancer cases. The authors found that statin use was associated with the reduced risk of liver cancer (RR = 0.67, 95%CI: 0.55-0.82, $P < 0.001$).

Applications

Statins may potentially be used for the therapy of liver cancer; however, more high quality studies, especially prospective intervention studies are required.

Peer review

The authors determined the association between statin use and the risk of liver cancer by a meta-analysis. This analysis involved 7 studies with 4725593 people and 9785 liver cancer cases from 2005 to 2013. The results showed a 36%-39% reduction in liver cancer risk when statins were used. This study is very interesting.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **Altekruse SF**, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009; **27**: 1485-1491 [PMID: 19224838 DOI: 10.1200/JCO.2008.20.7753]

3 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]

4 **El-Serag HB**, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460-468 [PMID: 14762783 DOI: 10.1053/j.gastro.2003.10.065]

5 **Lai MS**, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: A cohort study in high prevalence area of hepatitis virus infection. *Hepatology* 2006; **43**: 1295-1302 [PMID: 16729295 DOI: 10.1002/hep.21208]

6 **Bravi F**, Bosetti C, Tavani A, Bagnardi V, Gallus S, Negri E, Franceschi S, La Vecchia C. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. *Hepatology* 2007; **46**: 430-435 [PMID: 17580359 DOI: 10.1002/hep.21708]

7 **Fon Sing M**, Yang WS, Gao S, Gao J, Xiang YB. Epidemiological studies of the association between tea drinking and primary liver cancer: a meta-analysis. *Eur J Cancer Prev* 2011; **20**: 157-165 [PMID: 21403523 DOI: 10.1097/CEJ.0b013e3283447497]

8 **Lai SW**, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. *Am J Gastroenterol* 2012; **107**: 46-52 [PMID: 22085817 DOI: 10.1038/ajg.2011.384]

9 **Goldstein JL**, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990; **343**: 425-430 [PMID: 1967820 DOI: 10.1038/343425a0]

10 **Hsia J**, MacFadyen JG, Monyak J, Ridker PM. Cardiovascular event reduction and adverse events among subjects attaining low-density lipoprotein cholesterol & lt; 50 mg/dl with rosuvastatin. The JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin). *J Am Coll Cardiol* 2011; **57**: 1666-1675 [PMID: 21492764 DOI: 10.1016/j.jacc.2010.09.082]

11 **Bulbulia R**, Bowman L, Wallendszus K, Parish S, Armitage J, Peto R, Collins R. Effects on 11-year mortality and morbidity of lowering LDL cholesterol with simvastatin for about 5 years in 20,536 high-risk individuals: a randomised controlled trial. *Lancet* 2011; **378**: 2013-2020 [PMID: 22115874 DOI: 10.1016/S0140-6736(11)61125-2]

12 **Baigent C**, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, Wanner C, Krane V, Cass A, Craig J, Neal B, Jiang L, Hooi LS, Levin A, Agodoa L, Gaziano M, Kasiske B, Walker R, Massy ZA, Feldt-Rasmussen B, Krairittichai U, Ophascharoensuk V, Fellström B, Holdaas H, Tesar V, Wiecek A, Grobbee D, de Zeeuw D, Grönhagen-Riska C, Dasgupta T, Lewis D, Herrington W, Mafham M, Majoni W, Wallendszus K, Grimm R, Pedersen T, Tobert J, Armitage J, Baxter A, Bray C, Chen Y, Chen Z, Hill M, Knott C, Parish S, Simpson D, Sleight P, Young A, Collins R. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *Lancet* 2011; **377**: 2181-2192 [PMID: 21663949 DOI: 10.1016/S0140-6736(11)60739-3]

13 **Walley T**, Folino-Gallo P, Schwabe U, van Ganse E. Variations and increase in use of statins across Europe: data from administrative databases. *BMJ* 2004; **328**: 385-386 [PMID: 14962875 DOI: 10.1136/bmj.328.7436.385]

14 **Mück AO**, Seeger H, Wallwiener D. Inhibitory effect of statins on the proliferation of human breast cancer cells. *Int J Clin Pharmacol Ther* 2004; **42**: 695-700 [PMID: 15624286]

15 **Yasuda Y**, Shimizu M, Shirakami Y, Sakai H, Kubota M, Hata K, Hirose Y, Tsurumi H, Tanaka T, Moriwaki H. Pitavastatin inhibits azoxymethane-induced colonic preneoplastic lesions in C57BL/KsJ-db/db obese mice. *Cancer Sci* 2010; **101**: 1701-1707 [PMID: 20398056 DOI: 10.1111/j.1349-7006.2010.01579.x]

16 **Björkhem-Bergman L**, Acimovic J, Torndal UB, Parini P, Eriksson LC. Lovastatin prevents carcinogenesis in a rat model for liver cancer. Effects of ubiquinone supplementation. *Anticancer Res* 2010; **30**: 1105-1112 [PMID: 20530415]

17 **Chiu HF**, Ho SC, Chen CC, Yang CY. Statin use and the risk of liver cancer: a population-based case–control study. *Am J Gastroenterol* 2011; **106**: 894-898 [PMID: 21157439 DOI: 10.1038/ajg.2010.475]

18 **Tsan YT**, Lee CH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis B virus infection. *J Clin Oncol* 2012; **30**: 623-630 [PMID: 22271485 DOI: 10.1200/JCO.2011.36.0917]

19 **Bocci G**, Fioravanti A, Orlandi P, Bernardini N, Collecchi P, Del Tacca M, Danesi R. Fluvastatin synergistically enhances the antiproliferative effect of gemcitabine in human pancreatic cancer MIAPaCa-2 cells. *Br J Cancer* 2005; **93**: 319-330 [PMID: 16052215 DOI: 10.1038/sj.bjc.6602720]

20 **Kodach LL**, Jacobs RJ, Voorneveld PW, Wildenberg ME, Verspaget HW, van Wezel T, Morreau H, Hommes DW, Peppelenbosch MP, van den Brink GR, Hardwick JC. Statins augment the chemosensitivity of colorectal cancer cells inducing epigenetic reprogramming and reducing colorectal cancer cell 'stemness' via the bone morphogenetic protein pathway. *Gut* 2011; **60**: 1544-1553 [PMID: 21551187 DOI: 10.1136/gut.2011.237495]

21 **Wang W**, Le W, Cho DY, Hwang PH, Upadhyay D. Novel effects of statins in enhancing efficacy of chemotherapy in vitro in nasopharyngeal carcinoma. *Int Forum Allergy Rhinol* 2011; **1**: 284-289 [PMID: 22287433 DOI: 10.1002/alr.20039]

22 **Graf H**, Jüngst C, Straub G, Dogan S, Hoffmann RT, Jakobs T, Reiser M, Waggershauser T, Helmberger T, Walter A, Walli A, Seidel D, Goke B, Jüngst D. Chemoembolization combined with pravastatin improves survival in patients with hepatocellular carcinoma. *Digestion* 2008; **78**: 34-38 [PMID: 18797167 DOI: 10.1159/000156702]

23 **Emberson JR**, Kearney PM, Blackwell L, Newman C, Reith C, Bhala N, Holland L, Peto R, Keech A, Collins R, Simes J, Baigent C. Lack of effect of lowering LDL cholesterol on cancer: meta-analysis of individual data from 175,000 people in 27 randomised trials of statin therapy. *PLoS One* 2012; **7**: e29849 [PMID: 22276132 DOI: 10.1371/journal.pone.0029849]

24 **Bonovas S**, Filioussi K, Flordellis CS, Sitaras NM. Statins and the risk of colorectal cancer: a meta-analysis of 18 studies involving more than 1.5 million patients. *J Clin Oncol* 2007; **25**: 3462-3468 [PMID: 17687150 DOI: 10.1200/JCO.2007.10.8936]

25 **Clearfield M**, Downs JR, Weis S, Whitney EJ, Kruyer W, Shapiro DR, Stein EA, Langendorfer A, Beere PA, Gotto AM. Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS): efficacy and tolerability of long-term treatment with lovastatin in women. *J Womens Health Gend Based Med* 2001; **10**: 971-981 [PMID: 11788107 DOI: 10.1089/152460901317193549]

26 **Lang S**, Kleijnen J. Quality assessment tools for observational studies: lack of consensus. *Int J Evid Based Healthc* 2010; **8**: 247 [PMID: 21091888 DOI: 10.1111/j.1744-1609.2010.00195.x]

27 **Mallen C**, Peat G, Croft P. Quality assessment of observational studies is not commonplace in systematic reviews. *J Clin Epidemiol* 2006; **59**: 765-769 [PMID: 16828667 DOI: 10.1016/j.jclinepi.2005.12.010]

28 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188 [PMID: 3802833 DOI: 10.1016/0197-2456(86)90046-2]

29 **Mantel N,** Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959; **22**: 719-748 [PMID: 13655060]

30 **Schmidt FL**, Oh IS, Hayes TL. Fixed- versus random-effects models in meta-analysis: model properties and an empirical comparison of differences in results. *Br J Math Stat Psychol* 2009; **62**: 97-128 [PMID: 18001516]

31 **Villar J**, Mackey ME, Carroli G, Donner A. Meta-analyses in systematic reviews of randomized controlled trials in perinatal medicine: comparison of fixed and random effects models. *Stat Med* 2001; **20**: 3635-3647 [PMID: 11746343 DOI: 10.1002/sim.1096]

32 **Cochran WG.** The combination of estimates from different experiments. *Biometrics* 1954; **10**: 101-129 [DOI: 10.2307/3001666]

33 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560

[PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]
34 **Begg CB**, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**: 1088-1101 [PMID: 7786990]
35 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]
36 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670 DOI: 10.1001/jama.283.15.2008]
37 **El-Serag HB**, Johnson ML, Hachem C, Morgana RO. Statins are associated with a reduced risk of hepatocellular carcinoma in a large cohort of patients with diabetes. *Gastroenterology* 2009; **136**: 1601-1608 [PMID: 19208359 DOI: 10.1053/j.gastro.2009.01.053]
38 **Leung HW**, Chan AL, Lo D, Leung JH, Chen HL. Common cancer risk and statins: a population-based case-control study in a Chinese population. *Expert Opin Drug Saf* 2013; **12**: 19-27 [PMID: 23199231 DOI: 10.1517/14740338.2013.744392]
39 **Friis S**, Poulsen AH, Johnsen SP, McLaughlin JK, Fryzek JP, Dalton SO, Sørensen HT, Olsen JH. Cancer risk among statin users: a population-based cohort study. *Int J Cancer* 2005; **114**: 643-647 [PMID: 15578694 DOI: 10.1002/ijc.20758]
40 **Friedman GD**, Flick ED, Udaltsova N, Chan J, Quesenberry CP, Habel LA. Screening statins for possible carcinogenic risk: up to 9 years of follow-up of 361,859 recipients. *Pharmacoepidemiol Drug Saf* 2008; **17**: 27-36 [PMID: 17944002 DOI: 10.1002/pds.1507]
41 **Marelli C**, Gunnarsson C, Ross S, Haas S, Stroup DF, Cload P, Clopton P, DeMaria AN. Statins and risk of cancer: a retrospective cohort analysis of 45,857 matched pairs from an electronic medical records database of 11 million adult Americans. *J Am Coll Cardiol* 2011; **58**: 530-537 [PMID: 21777752 DOI: 10.1016/j.jacc.2011.04.015]
42 **Bielinska A**, Gluszko P. [Statins--are they potentially useful in rheumatology?]. *Pol Arch Med Wewn* 2007; **117**: 420-425 [PMID: 18062565]
43 **Mehta N**, Hordines J, Sykes D, Doerr RJ, Cohen SA. Low density lipoproteins and Lovastatin modulate the organ-specific transendothelial migration of primary and metastatic human colon adenocarcinoma cell lines in vitro. *Clin Exp Metastasis* 1998; **16**: 587-594 [PMID: 9932605]
44 **Wong WW**, Dimitroulakos J, Minden MD, Penn LZ. HMG-CoA reductase inhibitors and the malignant cell: the statin family of drugs as triggers of tumor-specific apoptosis. *Leukemia* 2002; **16**: 508-519 [PMID: 11960327 DOI: 10.1038/sj.leu.2402476]
45 **Park HJ**, Kong D, Iruela-Arispe L, Begley U, Tang D, Galper JB. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors interfere with angiogenesis by inhibiting the geranylgeranylation of RhoA. *Circ Res* 2002; **91**: 143-150 [PMID: 12142347 DOI: 10.1161/01.RES.0000028149.15986.4C]
46 **Thomsen RW**, Johnsen SP, Olesen AV, Mortensen JT, Bøggild H, Olsen J, Sørensen HT. Socioeconomic gradient in use of statins among Danish patients: population-based cross-sectional study. *Br J Clin Pharmacol* 2005; **60**: 534-542 [PMID: 16236044 DOI: 10.1111/j.1365-2125.2005.02494.x]
47 **Mamdani MM**, Tu K, Austin PC, Alter DA. Influence of socioeconomic status on drug selection for the elderly in Canada. *Ann Pharmacother* 2002; **36**: 804-808 [PMID: 11978155 DOI: 10.1345/aph.1A044]
48 **Lakshminarayana Reddy CN**, Vyjayanti VN, Notani D, Galande S, Kotamraju S. Down-regulation of the global regulator SATB1 by statins in COLO205 colon cancer cells. *Mol Med Rep* 2010; **3**: 857-861 [PMID: 21472326 DOI: 10.3892/mmr.2010.338]
49 **Bergman M**, Salman H, Djaldetti M, Bessler H. Statins as modulators of colon cancer cells induced cytokine secretion by human PBMC. *Vascul Pharmacol* 2011; **54**: 88-92 [PMID: 21440087 DOI: 10.1016/j.vph.2011.03.002]
50 **Menter DG**, Ramsauer VP, Harirforoosh S, Chakraborty K, Yang P, Hsi L, Newman RA, Krishnan K. Differential effects of pravastatin and simvastatin on the growth of tumor cells from different organ sites. *PLoS One* 2011; **6**: e28813 [PMID: 22216116 DOI: 10.1371/journal.pone.0028813]

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META-ANALYSIS

Relationship between apolipoprotein E gene polymorphism and total cholesterol level in patients with kidney diseases

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Abstract

AIM: To evaluate the association between apolipoprotein E (*apoE*) gene polymorphism and total cholesterol (TC) level in patients with kidney diseases.

METHODS: A predefined literature search was performed to collect data from the electronic databases of PubMed, Embase and the Cochrane Library and eligible relevant studies reporting the association of *apoE* gene polymorphism with TC level in patients with kidney diseases were recruited for meta-analysis.

RESULTS: Twenty-one studies were identified for the analysis of association between *apoE* gene polymorphism and TC level in patients with kidney disease. Subjects with E3E4 had a higher TC than those with E3E3 [weighted mean differences (WMD) = 2.14, P = 0.01] and subjects with E2E3 had a lower TC than those with E3E3 (WMD = -1.93, P = 0.01). Subjects with ε2 had a lower TC than those with ε3 (ε2 *vs* ε3: WMD = -1.23, P = 0.002; ε2 *vs* ε4: WMD = -2.77, P < 0.0001) and subjects with 3 had a lower TC than those with 4 (WMD = -0.79, P = 0.03).

CONCLUSION: Subjects with *apoE* E3E4 and ε4 had a higher TC level and subjects with *apoE* E2E3 and ε2 had a higher TC level in patients with kidney disease. However, more well-designed studies should be performed in the future to confirm these findings.

Key words: Apolipoprotein E; Gene polymorphism; Total cholesterol; Meta-analysis

Core tip: The available evidence for an association between apolipoprotein E (*apoE*) and total cholesterol (TC) in kidney disease is weak and has conflicting results. This meta-analysis based on 21 included studies indicated that subjects with *apoE* E3E4 and 4 had a higher TC level and subjects with *apoE* E2E3 and ε2 had a higher TC level in patients with kidney disease.

Zhou TB, Jiang ZP, Yin SS, Qin YH. Relationship between apolipoprotein E gene polymorphism and total cholesterol level in patients with kidney diseases. *World J Meta-Anal* 2013; 1(3): 138-146 Available from: URL: http://www.wjgnet.com/2308-3840/full/v1/i3/138.htm DOI: http://dx.doi.org/10.13105/wjma.v1.i3.138

INTRODUCTION

Apolipoprotein E (apoE) is an important protein of the lipoprotein transport system and plays an important role in lipoprotein metabolism and lipid homeostasis[1-3]. It is

associated with the metabolism of total cholesterol (TC) and triglyceride[4,5]. ApoE, a 229-amino-acid polypeptide, is classified into three major isoforms (ε2, ε3 and ε4) according to the differences in amino acids at positions 112 and 158, forming six genotypes: E2E2, E2E3, E2E4, E3E3, E3E3 and E4E4[6,7]. ε3 and E3E3 are the wild type of apoE and ε2, ε4, E2E2, E2E3, E2E4, E3E3 and E4E4 are the mutation type of apoE.

The represent one of the major systems that maintain the body homeostasis[8]. Abnormalities of lipid metabolism and homeostasis are commonly present in patients with kidney disease[9], such as nephrotic syndrome (NS). Most kidney diseases are associated with elevated serum/plasma concentration and the impaired clearance of very low density lipoprotein and their atherogenic remnants. Nephrotic dyslipidemia is a risk factor for the development of systemic atherosclerosis, it may aggravate the glomerulosclerosis lesion and enhance the progression of glomerular disease[10]. apoE lipoprotein plays an important role in lipid homeostasis and kidney diseases.

In renal tissue, apoE is mainly synthesized by mesangial cells under normal physiological conditions[11,12]. Investigators reported that apoE had a protective role for the kidney[13,14]. Some other investigations found that the apoE expression in renal glomerulus was elevated when compared with that of the normal group[15-19]. There were some investigations reporting that apoE polymorphism was associated with renal diseases. In our previous study[20], we reported that the *apoE* gene expression was associated with the NS susceptibility in experimental studies and the apoE ε3ε3, ε3ε4, ε3 and ε4 were associated with the onset of NS in human studies. Hayakawa *et al*[21] carried out an investigation on a patient with apoE ε3ε3 variant without lipoprotein glomerulopathy and illustrated that not all apoE variants resulted in lipoprotein glomerulopathy and that the location of mutations in the apoE protein was one of the important determinants for the development of lipoprotein glomerulopathy. Luo *et al*[22] reported that apoE mutation was associated with the lipoprotein glomerulopathy risk and found that the apoE mutation could cause a marked molecular conformational change of apoE and thus impaired its binding ability to lipids.

Some reports show that *apoE* gene polymorphism is associated with the expression of TC. However, some studies found that *apoE* gene polymorphism was not associated with the expression of TC. The available evidence for an association between apoE and TC in kidney disease is weak due to paucity of data or disagreements among the reported studies. Thus, evidence from a meta-analysis may be more powerful compared to single studies[23]. To date, no meta-analysis exists that determines the association between *apoE* gene polymorphism and TC level in patients with kidney diseases. This meta-analysis was performed to investigate the association of the *apoE* gene polymorphism with the TC level in patients with kidney diseases.

MATERIALS AND METHODS

Search strategy

The relevant studies were screened from the search engines of PubMed, Embase and the Cochrane Library on March 1, 2012. "(apoE AND apolipoprotein E) AND (renal OR kidney)" was used in PubMed, Embase and the Cochrane Library. We also extended our search spectrum to the "related articles" and the bibliographies of all retrieved studies. If multiple publications from the same study group using the same data were reported, we only recruited the most complete study for our analysis.

Inclusion and exclusion criteria

Inclusion criteria: (1) the study had to be about kidney diseases; (2) provide detailed gene distribution of apoE; and (3) report on the level of TC.

Exclusion criteria: (1) Editorials, reviews, case reports; (2) data from multiple publications; (3) investigating the association of other genes in kidney diseases; and (4) investigating the role of apoE in diseases other than kidney diseases.

Data extraction and synthesis

The following information was extracted from each study independently by the investigators: first author's surname, year of publication, location of study, patient type, level of TC and the number of subjects. Study type, comparisons of *apoE* gene polymorphism and methodological quality assessment outcomes were also extracted. The results were compared and disagreements were resolved by discussion.

Statistical analysis

Available data was entered into Cochrane Review Manager (RevMan, Version 5) and analyzed. The pooled statistic was counted using the fixed effects model but a random effects model was conducted when the *P* value of heterogeneity test was less than 0.1. Results were expressed with weighted mean differences (WMD) for continuous data and 95%CI were also counted. $P < 0.05$ was required for the overall WMD to be deemed statistically significant. I^2 was used to test the heterogeneity between the included studies. The Begg adjusted rank correlation test[24] and the Egger regression asymmetry test[25] were used for exploring publication bias ($P < 0.1$ was considered significant) when the number of the included studies was more than fifteen.

RESULTS

Study characteristics for the relationship between apoE gene polymorphism with TC expression

Twenty-one studies were included in the meta-analysis for the relationship between *apoE* gene polymorphism with TC expression (Figure 1 and Table 1). One study[26] was for the comparison of E2E2 *vs* E3E3. Eleven reports[26-36] were recruited into the study of E2E3 *vs* E3E3 (including 13 comparisons). Three reports[26,27,29] were recruited into the study of E2E4 *vs* E3E3 (including 3 comparisons). 11 reports[26-36] were recruited into the study of E3E4 *vs*

Table 1 The detailed characteristics of included studies

Author	Study type	Location of study	Ethnicity	Patient type	Number of subjects	Comparisons of *apoE* gene polymorphism	Genotyping method reported	Blinding of genotyping
Feussner *et al*[26]	Prospective	Germany	Caucasian	ESRD	141 males and 104 females	E2E2, E2E3, E2E4, E3E3, E3E4, E4E4	No	Not mention
Eggertsen *et al*[29]	Prospective	Sweden	Caucasian	ESRD: 19 cases glomerulonephritis, 11 cases diabetic nephropathy, nine cases interstitial nephritis	25 males and 26 females	E2E3, E2E4, E3E3, E3E4, E4E4	Yes	Not mention
Oda *et al*[28]	Prospective	Japan	Asian	107 with GN and 399 with ESRD	Patients with GN consisted of 42 men and 65 women	E2E3, E3E3, E3E4, ε2, ε3, ε4	Yes	Not mention
Lim *et al*[38]	Prospective	China	Asian	ESRD: 85 patients with chronic GN, 30 patients with DN, 18 patients with chronic pyelonephritis, 3 patients with PKD	96 males and 60 females.	ε4, non-ε4	Yes	Not mention
Kimura *et al*[39]	Prospective	Japan	Asian	DN	88 men and 90 women	ε4, non-ε4	Yes	Not mention
Werle *et al*[30]	Prospective	Germany	Caucasian	DN	159 men and 129 women	E2E3, E3E3, ε2, ε3, ε4	Yes	Not mention
Imura *et al*[31]	Prospective	Japan	Asian	ESRD	287 men and 206 women	E2E3, E3E3, E3E4, ε2, ε3, ε4	Yes	Not mention
Xiang *et al*[43]	Prospective	China	Asian	DN	26 men and 20 women	ε2, ε3	Yes	Not mention
Güz *et al*[32]	Prospective	Turkey	Caucasian	ESRD: GN (107 cases), hypertension nephropathy (37 cases), unknown (36 cases), pyelonephritis (29 cases), amyloidosis (20 cases), DN (15 cases), obstructive uropathy (9 cases), PKD (7 cases), toxic nephropathy (6 cases), and Alport's syndrome (3 cases)	149 men and 112 women	E2E3, E3E3, E3E4, ε2, ε3, ε4	Yes	Not mention
Zahálková *et al*[34]	Prospective	Czech	Caucasian	ESRD	53 males and 34 females	E2E3, E3E3, E3E4, ε2, ε3, ε4	Yes	Not mention
Lehtinen *et al*[40]	Prospective	Finland	Caucasian	DN	Not mention	ε4, non-ε4	Yes	Not mention
Kahraman *et al*[42]	Prospective	Turkey	Caucasian	Renal transplant recipients	80 males and 38 females	ε2, ε3, ε4	Yes	Not mention
Joss *et al*[36]	Prospective	United Kingdom	Caucasian	DN	Not mention	E2E3, E3E3, E3E4, ε2, ε3, ε4	Yes	Not mention
Maluf *et al*[45]	Prospective	United States	Mix	Renal transplant recipients	21 males and 18 females	ε2, ε3, ε4	Yes	Not mention
Arikan *et al*[35]	Prospective	Turkey	Caucasian	ESRD	84 males and 60 females	E2E3, E3E3, E3E4, ε2, ε3, ε4	Yes	Not mention
Kwon *et al*[41]	Retrospective	Korea	Asian	DN	32 males and 62 females	ε2, ε3, ε4	No	Not mention
Leiva *et al*[46]	Retrospective	Chile	South America	DN	53 males and 32 females	ε3, ε4	Yes	Not mention
Ma *et al*[37]	Prospective	China	Asian	DN	146 males and 259 females	ε2, non-ε2	Yes	Not mention
Erdogan *et al*[33]	Prospective	Turkey	Caucasian	DN	19 males and 27 females	E2E3, E3E3, E3E4, ε2, ε3, ε4	Yes	Not mention
Hu *et al*[27]	Prospective	China	Asian	MCNS	176 males and 74 females	E2E2, E2E3, E2E4, E3E3, E3E4, E4E4, ε2, ε3, ε4	Yes	Not mention
Li *et al*[44]	Prospective	China	Asian	Renal transplant recipients	59 males and 46 females	ε2, ε3, ε4	Yes	Not mention

ESRD: End-stage renal disease; GN: Glomerulonephritis; DN: Diabetic nephropathy; PKD: Polycystic kidney disease; MCNS: Minimal change nephrotic syndrome.

E3E3 (including 13 comparisons). Three reports[26,27,29] were recruited into the study of E4E4 *vs* E3E3 (including 3 comparisons). Two reports[37] were included for the meta-analysis of ε2 *vs* non-ε2 (including 2 compari-

Table 2 Meta-analysis of the association of *apoE* gene polymorphism with total cholecterol level

Genetic comparisons	*Q* test	*P* contrasts	Model seclected	Weighted mean differences (95%CI)	*P* value
E2E2 *vs* E3E3	1	-	Fixed	109.00 (-32.07-250.07)	0.13
E2E3 *vs* E3E3	13	< 0.00001	Random	-1.93 (-3.39--0.46)	0.01
E2E4 *vs* E3E3	3	0.48	Random	-2.48 (-4.23--0.72)	0.006
E3E4 *vs* E3E3	13	< 0.00001	Random	2.14 (0.46-3.83)	0.01
E4E4 *vs* E3E3	3	0.02	Random	0.30 (-4.25-4.84)	0.90
ε2 *vs* non-ε2	2	0.57	Fixed	-0.25 (-0.43--0.08)	0.005
ε4 *vs* non-ε4	4	0.0001	Random	0.20 (-1.42-1.83)	0.81
ε2 *vs* ε3	20	< 0.00001	Random	-1.23 (-1.99--0.46)	0.002
ε3 *vs* ε4	20	< 0.00001	Random	-0.79 (-1.50--0.08)	0.03
ε2 *vs* ε4	19	< 0.00001	Random	-2.77 (-4.05--1.49)	< 0.0001

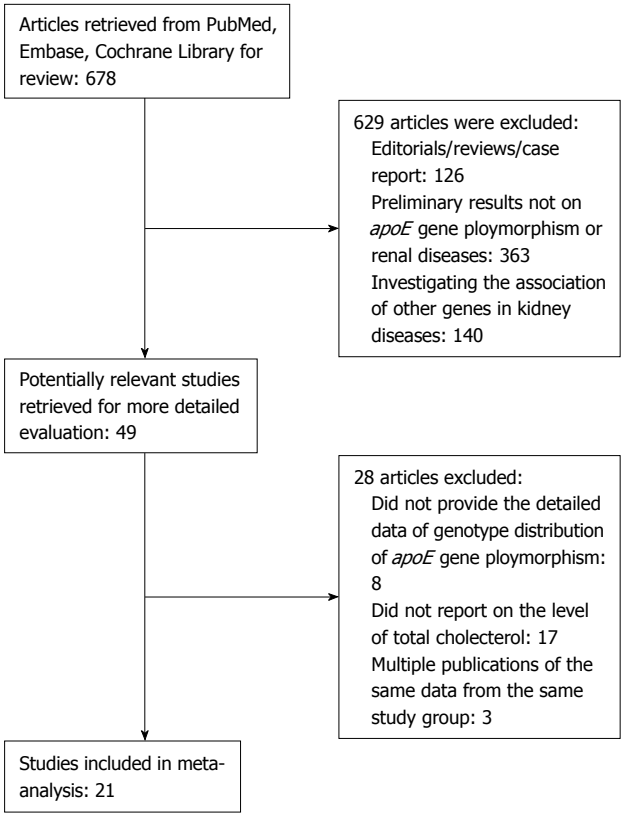


Figure 1 Flow diagram for this meta-analysis.

sons). Three studies[38-40] were recruited into our meta-analysis for the comparison of ε4 with non-ε4 (including 4 comparisons). 13 studies[27,28,30-36,41-45] were included into the study of ε2 *vs* ε3 (including 20 comparisons). Fourteen studies[27,28,30-36,41,42,44-46] were included into the study of ε4 *vs* ε4 (including 20 comparisons). Thirteen studies[27,28,30-36,41,42,44,45] were included into the study of ε2 *vs* ε4 (including 19 comparisons).

Association of apoE gene polymorphism with TC expression

In this meta-analysis, the number of included studies for some comparisons was more than ten (E2E3 *vs* E3E3, E3E4 *vs* E3E3, ε2 *vs* ε3, ε4 *vs* ε3, ε2 *vs* ε4) and the number of included studies for some comparisons was less ten (E2E2 *vs* E3E3, E2E4 *vs* E3E3, E4E4 *vs* E3E3, ε2 *vs* non-ε2, ε4 *vs* non-ε4). Those results from less than ten might be less robust. We presented those results independently.

Subjects with E3E4 had a higher TC than those with E3E3 (Figure 2A and Table 2) and subjects with E2E3 had a lower TC than those with E3E3 (Figure 2B and Table 2). Subjects with ε2 had a lower TC than those with ε3 or ε4 (Figure 2C for ε3 and Figure 2D for ε4; Table 2) and subjects with ε4 had a higher TC than those with ε3 (Figure 2E and Table 2). It seemed that E3E4 and ε4 were associated with higher level of TC and E2/E3 and ε2 were associated with lower level of TC. The number of included studies for some comparisons was more than ten and those results might be robust to some extent.

Subjects with E2E4 had a much lower TC level when compared with those with E3E3. Subjects with ε2 had a lower level of TC than those with non-ε2. Subjects with E2E2 or E4E4 had a slightly higher TC than those with E3E3, although there was no statistical difference. Subjects with 4 had a similar level of TC than those with non-ε4 (Table 2). It seemed that E2E4 and ε2 was associated with lower level of TC. The number of included studies for some comparisons was less than ten and more studies should be performed in the future.

Publication bias test for the association of apoE gene polymorphism with TC expression

There was a publication bias test for the comparisons of E2E3 *vs* E3E3, E3E4 *vs* E3E3, ε2 *vs* ε3, ε4 *vs* ε3, ε2 *vs* ε4. There was no publication bias for the comparisons of E2E3 *vs* E3E3, E3E4 *vs* E3E3, ε2 *vs* ε3, ε4 *vs* ε3, ε2 *vs* ε4 (E2E3 *vs* E3E3: Begg $P = 0.951$, Egger $P = 0.936$; E3E4 *vs* E3E3: Begg $P = 0.951$, Egger $P = 0.710$; ε2 *vs* ε3: Begg $P = 0.871$, Egger $P = 0.970$; ε4 *vs* ε3: Begg $P=0.315$, Egger $P = 0.251$; ε2 *vs* ε4: Begg $P=0.294$, Egger $P = 0.495$).

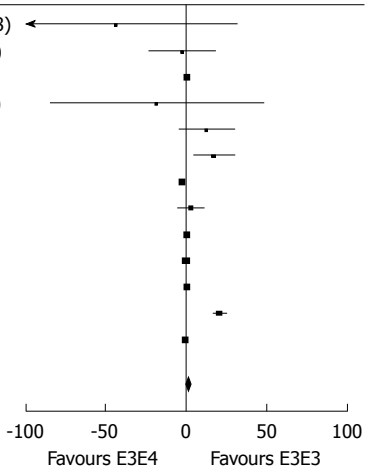
DISCUSSION

High serum TC level is an important characteristic for many kidney diseases, such as NS[47], glomerulonephritis[48], end-stage kidney disease requiring dialysis[49], *etc*. Raised TC is a consequence of kidney disease but may accelerate progression to end-stage kidney disease. In our meta-analysis, we found that subjects with E3E4 had a higher TC than those with E3E3 and subjects with E2E3

A

Study or subgroup	E3E4 Mean	E3E4 SD	E3E4 Total	E3E3 Mean	E3E3 SD	E3E3 Total	Weight (%)	Mean difference IV, Random, 95%CI
Arikan *et al*[35], 2007	223.4	85.6	5	267.2	39.9	54	0.0	-43.80 (-119.58-31.98)
Arikan *et al*[35], 2007	194.8	20.7	7	197.3	50.6	53	0.6	-2.50 (-23.01-18.01)
Eggertsen *et al*[29], 1997	7.36	1.21	14	6.49	1.5	30	14.5	0.87 (0.04-1.70)
Erdogan *et al*[33], 2009	194	73.386	5	212.14	46.824	36	0.1	-18.14 (-84.26-47.98)
Feussner *et al*[26], 1992	222	55	54	209	58	143	0.9	13.00 (-4.48-30.48)
Güz *et al*[32], 2000	163.96	32.1	28	146.5	34.51	200	1.5	17.46 (4.64-30.28)
Hu *et al*[27], 2010	7.1	2.2	10	9.7	3.8	195	13.5	-2.60 (-4.06--1.14)
Imura *et al*[31], 1999	164	34	90	161	34	350	3.5	3.00 (-4.88-10.88)
Joss *et al*[36], 2005	5.9	2.1	17	5.5	1.2	49	14.2	0.40 (-0.65-1.45)
Oda *et al*[28], 1997	4.81	1.01	18	4.39	0.85	80	14.8	0.42 (-0.08-0.92)
Oda *et al*[28], 1997	6.07	1.13	5	5.19	1.35	14	13.9	0.88 (-0.34-2.10)
Werle *et al*[30], 1998	241	9.5	23	220	4.3	72	8.1	21.00 (16.99-25.01)
Zahálková *et al*[34], 2002	5.1	1.46	11	5.2	1.36	57	14.3	-0.10 (-1.03-0.83)
Total (95%CI)			287			1333	100.0	2.14 (0.46-3.83)

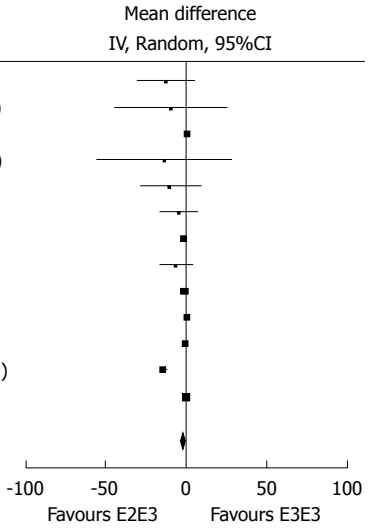
Heterogeneity: Tau^2 = 4.91; χ^2 = 131.34, df = 12 (P < 0.00001); I^2 = 91%
Test for overall effect: Z = 2.50 (P = 0.01)



B

Study or subgroup	E3E4 Mean	E3E4 SD	E3E4 Total	E3E3 Mean	E3E3 SD	E3E3 Total	Weight (%)	Mean difference IV, Random, 95%CI
Arikan *et al*[35], 2007	154.7	23.5	11	167.2	39.9	54	0.7	-12.50 (-30.00-5.00)
Arikan *et al*[35], 2007	187.7	42.5	7	197.3	50.6	53	0.2	-9.60 (-43.90-24.70)
Eggertsen *et al*[29], 1997	6.9	0.99	2	6.49	1.5	30	13.4	0.41 (-1.06-1.88)
Erdogan *et al*[33], 2009	198.67	34.53	3	212.14	46.824	36	0.1	-13.47 (-55.43-28.49)
Feussner *et al*[26], 1992	199	44	29	209	58	143	0.6	-10.00 (-28.62-8.62)
Güz *et al*[32], 2000	141.9	31.56	33	146.5	34.51	200	1.4	-4.60 (-16.38-7.18)
Hu *et al*[27], 2010	8.7	2.7	22	9.7	3.8	195	13.9	-1.00 (-2.25-0.25)
Imura *et al*[31], 1999	155	36	53	161	34	350	1.8	-6.00 (-16.33-4.33)
Joss *et al*[36], 2005	4.9	0.9	20	5.5	1.2	49	15.2	-0.60 (-1.12--0.08)
Oda *et al*[28], 1997	6.27	3.22	8	5.22	1.19	79	11.3	1.05 (-1.20-3.30)
Oda *et al*[28], 1997	4.39	0.67	16	4.39	0.85	80	15.3	0.00 (-0.38-0.38)
Werle *et al*[30], 1998	180	6.4	33	194	3.5	92	11.2	-14.00 (-16.30--11.70)
Zahálková *et al*[34], 2002	5.49	0.78	9	5.2	1.36	57	15.0	0.29 (-0.33-0.91)
Total (95%CI)			246			1418	100.0	-1.93 (-3.39--0.46)

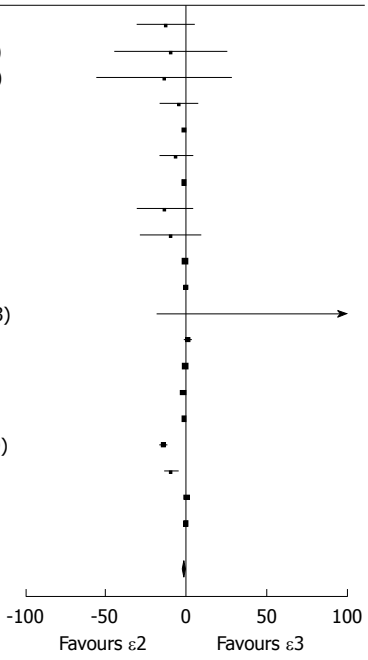
Heterogeneity: Tau^2 = 3.62; χ^2 = 152.50, df = 12 (P < 0.00001); I^2 = 92%
Test for overall effect: Z = 2.58 (P = 0.010)



C

Study or subgroup	ε2 Mean	ε2 SD	ε2 Total	ε3 Mean	ε3 SD	ε3 Total	Weight (%)	Mean difference IV, Random, 95%CI
Arikan *et al*[35], 2007	154.7	23.5	11	167.2	39.9	54	0.2	-12.50 (-30.00-5.00)
Arikan *et al*[35], 2007	187.7	42.5	7	197.3	50.6	53	0.0	-9.60 (-43.90-24.70)
Erdogan *et al*[33], 2009	198.67	34.53	3	212.14	46.824	36	0.0	-13.47 (-55.43-28.49)
Güz *et al*[32], 2000	141.9	31.56	33	146.5	34.51	200	0.4	-4.60 (-16.38-7.18)
Hu *et al*[27], 2010	8.7	2.5	18	9.7	3.8	211	8.0	-1.00 (-2.26-0.26)
Imura *et al*[31], 1999	155	36	53	161	34	350	0.5	-6.00 (-16.33-4.33)
Joss *et al*[36], 2005	4.9	0.9	20	5.5	1.2	49	9.8	-0.60 (-1.12--0.08)
Kahraman *et al*[42], 2004	209	25	12	222	43	92	0.2	-13.00 (-29.65-3.65)
Kwon *et al*[41], 2007	187.2	21.8	8	196.6	32.2	36	0.2	-9.40 (-27.81-9.01)
Li *et al*[44], 2010	3.81	0.6	17	3.84	0.54	77	10.1	-0.03 (-0.34-0.28)
Li *et al*[44], 2010	5.87	1.88	17	5.5	1.42	77	8.9	0.37 (-0.58-1.32)
Maluf *et al*[45], 2005	324	175.5	8	217.4	116.5	78	0.0	106.60 (-17.73-230.93)
Oda *et al*[28], 1997	6.27	3.22	8	5.22	1.19	79	5.5	1.05 (-1.20-3.30)
Oda *et al*[28], 1997	4.39	0.67	16	4.39	0.85	80	10.0	0.00 (-0.38-0.38)
Oda *et al*[28], 1997	4.04	0.5	4	5.19	1.35	14	9.1	-1.15 (-2.01--0.29)
Ukkola *et al*[59], 2009	4.8	0.9	46	5.7	1.1	301	10.1	-0.90 (-1.19--0.61)
Werle *et al*[30], 1998	180	6.4	33	194	3.5	92	5.3	-14.00 (-16.30--11.70)
Werle *et al*[30], 1998	211	9.7	22	220	4.3	72	2.5	-9.00 (-13.17--4.83)
Xiang *et al*[43], 1999	5.7	1	10	4.7	1.2	28	9.3	1.00 (0.24-1.76)
Zahálková *et al*[34], 2002	5.49	0.78	9	5.2	1.36	57	9.6	0.29 (-0.33-0.91)
Total (95%CI)			355			2036	100.0	-1.23 (-1.99--0.46)

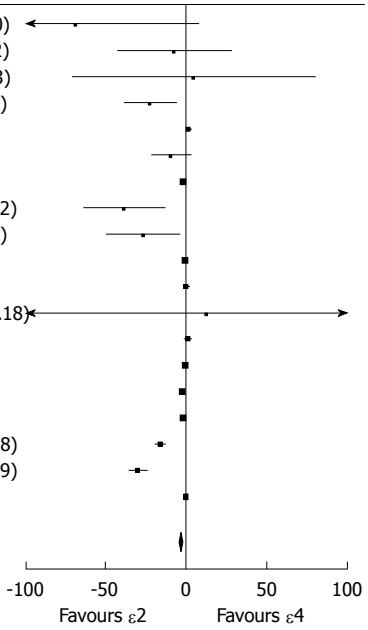
Heterogeneity: Tau^2 = 1.49; χ^2 = 208.48, df = 19 (P < 0.00001); I^2 = 91%
Test for overall effect: Z = 2.13 (P = 0.002)



D

Study or subgroup	ε2 Mean	ε2 SD	ε2 Total	ε4 Mean	ε4 SD	ε4 Total	Weight (%)	Mean difference IV, Random, 95%CI
Arikan *et al*[35], 2007	154.7	23.5	11	223.4	85.6	5	0.0	-68.70 (-145.00-7.60)
Arikan *et al*[35], 2007	187.7	42.5	7	194.8	20.7	7	0.1	-7.10 (-42.12-27.92)
Erdogan *et al*[33], 2009	198.67	34.53	3	194	73.386	5	0.0	4.67 (-70.59-79.93)
Güz *et al*[32], 2000	141.9	31.56	33	163.96	32.1	28	0.6	-22.06 (-38.10--6.02)
Hu *et al*[27], 2010	8.7	2.5	18	7.3	2	21	9.3	1.40 (-0.04-2.84)
Imura *et al*[31], 1999	155	36	53	164	34	90	1.0	-9.00 (-20.97-2.97)
Joss *et al*[36], 2005	4.9	0.9	20	5.9	2.1	17	9.8	-1.00 (-2.07-0.07)
Kahraman *et al*[42], 2004	209	25	12	247	40	14	0.2	-38.00 (-63.28--12.72)
Kwon *et al*[41], 2007	187.2	21.8	8	213.4	30.8	13	0.3	-26.20 (-48.75--3.65)
Li *et al*[44], 2010	3.81	0.6	17	4.33	0.68	11	10.4	-0.52 (-1.01--0.03)
Li *et al*[44], 2010	5.87	1.88	17	5.45	1.3	11	9.7	0.42 (-0.76-1.60)
Maluf *et al*[45], 2005	324	175.5	8	310.8	149	12	0.0	13.20 (-134.78-161.18)
Oda *et al*[28], 1997	6.27	3.22	8	5.29	1.38	20	7.8	0.98 (-1.33-3.29)
Oda *et al*[28], 1997	4.39	0.67	16	4.81	1.01	18	10.0	-0.42 (-0.99-0.15)
Oda *et al*[28], 1997	4.04	0.5	4	6.07	1.13	5	9.8	-2.03 (-3.14--0.92)
Ukkola *et al*[59], 2009	4.8	0.9	46	5.8	1	179	10.5	-1.00 (-1.30--0.70)
Werle *et al*[30], 1998	180	6.4	33	196	5.7	33	6.8	-16.00 (-18.92--13.08)
Werle *et al*[30], 1998	211	9.7	22	241	9.5	23	3.5	-30.00 (-35.61--24.39)
Zahálková *et al*[34], 2002	5.49	0.78	9	5.1	1.46	11	9.9	0.39 (-0.61-1.39)
Total (95%CI)			345			523	100.0	-2.77 (-4.05--1.49)

Heterogeneity: Tau^2 = 4.04; χ^2 = 263.05, df = 18 (P < 0.00001); I^2 = 93%
Test for overall effect: Z = 4.24 (P < 0.0001)



E

Study or subgroup	ε3 Mean	ε3 SD	ε3 Total	ε4 Mean	ε4 SD	ε4 Total	Weight (%)	Mean difference IV, Random, 95%CI
Arikan *et al*[35], 2007	167.2	39.9	54	223.4	85.6	5	0.0	-56.20 (-131.98-19.58)
Arikan *et al*[35], 2007	197.3	50.6	53	194.8	20.7	7	0.1	2.50 (-18.01-23.01)
Erdogan *et al*[33], 2009	212.14	46.824	36	194	73.386	5	0.0	18.14 (-47.98-84.26)
Güz *et al*[32], 2000	146.5	34.51	200	163.96	32.1	28	0.3	-17.46 (-30.28--4.64)
Hu *et al*[27], 2010	9.7	3.8	211	7.3	2	21	8.5	2.40 (1.40-3.40)
Imura *et al*[31], 1999	161	34	350	164	34	90	0.7	-3.00 (-10.88-4.88)
Joss *et al*[36], 2005	5.5	1.2	49	5.9	2.1	17	8.3	-0.40 (-1.45-0.65)
Kahraman *et al*[42], 2004	222	43	92	247	40	14	0.1	-25.00 (-47.72--2.28)
Kwon *et al*[41], 2007	196.6	32.2	36	213.4	30.8	13	0.1	-16.80 (-36.57-2.97)
Leiva *et al*[46], 2007	4.5	1.2	102	5.2	1.1	9	9.2	-0.70 (-1.46-0.06)
Li *et al*[44], 2010	3.84	0.54	77	4.33	0.68	11	9.9	-0.49 (-0.91--0.07)
Li *et al*[44], 2010	5.5	1.42	77	5.45	1.3	11	9.0	0.05 (-0.78-0.88)
Maluf *et al*[45], 2005	217.4	116.5	78	310.8	149	12	0.0	-93.40 (-181.58--5.22)
Oda *et al*[28], 1997	5.22	1.19	79	5.29	1.38	20	9.4	-0.07 (-0.73-0.59)
Oda *et al*[28], 1997	4.39	0.85	80	4.81	1.01	18	9.7	-0.42 (-0.92-0.08)
Oda *et al*[28], 1997	5.19	1.35	14	6.07	1.13	5	7.9	-0.88 (-2.10-0.34)
Ukkola *et al*[59], 2009	5.7	1.1	301	5.8	1	179	10.2	-0.10 (-0.29-0.09)
Werle *et al*[30], 1998	194	3.5	92	196	5.7	33	5.5	-2.00 (-4.07-0.07)
Werle *et al*[30], 1998	220	4.3	72	241	9.5	23	2.4	-21.00 (-25.01--16.99)
Zahálková *et al*[34], 2002	5.2	1.36	57	5.1	1.46	11	8.7	0.10 (-0.83-1.03)
Total (95%CI)			2110			532	100.0	-0.79 (-1.50--0.08)

Heterogeneity: Tau^2 = 1.27; χ^2 = 161.50, df = 19 (P < 0.00001); I^2 = 88%
Test for overall effect: Z = 2.19 (P = 0.03)

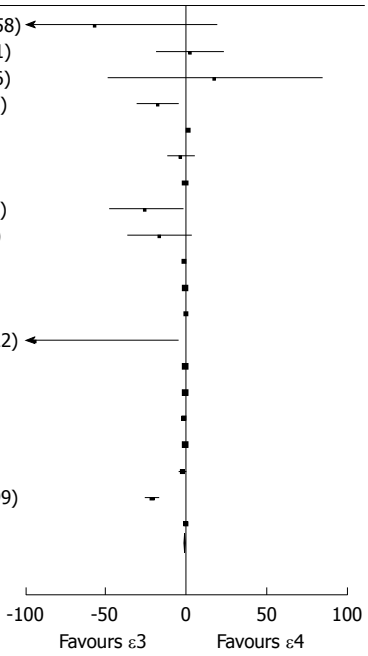


Figure 2 Association of *apoE* gene polymorphism with total cholesterol level. A: Using the comparison of E3E4 *vs* E3E3; B Using the comparison of E2E3 *vs* E3E3; C: Using the comparison of ε2 *vs* ε3; D: Using the comparison of ε2 *vs* ε4; E: Using the comparison of ε3 *vs* ε4.

had a lower TC than those with E3E3. Subjects with ε2 had a lower TC than those with ε3 or ε4, and subjects with ε4 had a higher TC than those with ε3. There was no publication bias test for the comparisons of E2E3 *vs* E3E3, E3E4 *vs* E3E3, ε2 *vs* ε3, ε4 *vs* ε3, ε2 *vs* ε4. E3E4 and ε4 were associated with higher level of TC and E2/E3 and ε2 were associated with lower level of TC. The conclusions for those comparisons were robust to some extent. Toms *et al*[50] reported that the ε2 allele was associated with the lowest and ε4 allele with the highest level of TC in rheumatoid arthritis patients. Alvim *et al*[51] reported that the ε4 allele was associated with higher TC value in healthy urban Brazilian individuals. Rahimi *et al*[52] found that ε4 allele resulted in a significant increase in the level of TC in sickle cell disease. Smart *et al*[53] reported that significantly higher TC was observed in apoE ε4 carriers compared to E3E3 homozygotes and ε2 carriers in 882 Greek children. The results from those studies

mentioned above were similar to our results in this meta-analysis.

The roles of apoE in diseases were also complicated. Many studies reported that apoE could play a protective role against diseases. apoE can play an antioxidant role[54], has been demonstrated to play an important role in providing protection against mesangial cell injury[55] and also appears to be involved in the repair response to tissue injury; for example, markedly increased amounts of apoE are found at sites of peripheral nerve injury and regeneration[56]. apoE deficiency in mice leads to the development of atherosclerosis and re-expression of the protein reduces the extent of the disease[57]. It appears that the increased apoE is a protective factor against disease progression. However, more studies need to be performed to explore the role of apoE in diseases.

There were some meta-analyses to evaluate the relationship between *apoE* gene polymorphism and TC level. Anthopoulos *et al*[58] performed a meta-analysis to detect the association of *apoE* gene polymorphism with TC level in patients with type 2 diabetes and the meta-regression analysis provided some weak evidence that the risk conferred by ε2 allele was mediated through altering serum TC level. Our study reported the association of *apoE* gene polymorphism with increased TC levels in patients with kidney diseases and showed that *apoE* gene polymorphism was associated with the increased levels of TC in patients with kidney disease.

In this meta-analysis, it was difficult to conduct the methodological quality assessment of included studies for the reason that the information in the included studies was few. More well-designed studies should be performed in the future.

In conclusion, our meta-analysis shows that *apoE* gene polymorphism is associated with increased levels of TC. However, larger studies should be performed in the future to confirm this association.

COMMENTS

Background

Apolipoprotein E (apoE), one of the major plasma lipoproteins, plays a major role in the transport and metabolism of lipids by acting as a ligand. *apoE* gene contains three potential alleles: ε2, ε3 and ε4, forming six genotypes: E2E2, E2E3, E2E4, E3E3, E3E3 and E4E4. An association between *apoE* gene polymorphism and total cholesterol (TC) level is still controversial.

Innovations and breakthroughs

To date, no meta-analysis exists that determines the association between the *apoE* gene polymorphism and TC level in patients with kidney diseases. This meta-analysis was performed to investigate the association of *apoE* gene polymorphism with the TC level in patients with kidney diseases.

Peer review

This paper performed a meta-analysis to investigate the relationship between *apoE* gene polymorphism and TC level in patients with renal diseases. The paper presents an interesting topic.

REFERENCES

1 **Annema W**, Dikkers A, Freark de Boer J, Gautier T, Rensen PC, Rader DJ, Tietge UJ. ApoE promotes hepatic selective uptake but not RCT due to increased ABCA1-mediated cholesterol efflux to plasma. *J Lipid Res* 2012; **53**: 929-940 [PMID: 22383685 DOI: 10.1194/jlr.M020743]

2 **Georgiadou D**, Chroni A, Vezeridis A, Zannis VI, Stratikos E. Biophysical analysis of apolipoprotein E3 variants linked with development of type III hyperlipoproteinemia. *PLoS One* 2011; **6**: e27037 [PMID: 22069485 DOI: 10.1371/journal.pone.0027037]

3 **Zhou TB**, Qin YH, Lei FY, Su LN, Zhao YJ, Huang WF. apoE expression in glomerulus and correlation with glomerulosclerosis induced by adriamycin in rats. *Ren Fail* 2011; **33**: 348-354 [PMID: 21401362 DOI: 10.3109/0886022X.2011.559680]

4 **Losonczi E**, Bencsik K, Fricska Nagy Z, Honti V, Szalczer E, Rajda C, Illés Z, Mátyás K, Rózsa C, Csépány T, Füvesi J, Vécsei L. APOE epsilon status in Hungarian patients with primary progressive multiple sclerosis. *Swiss Med Wkly* 2010; **140**: w13119 [PMID: 21110240 DOI: 10.4414/smw.2010.13119]

5 **Brito DD**, Fernandes AP, Gomes KB, Coelho FF, Cruz NG, Sabino AP, Cardoso JE, Figueiredo-Filho PP, Diamante R, Norton CR, Sousa MO. Apolipoprotein A5-1131T& gt; C polymorphism, but not APOE genotypes, increases susceptibility for dyslipidemia in children and adolescents. *Mol Biol Rep* 2011; **38**: 4381-4388 [PMID: 21132386 DOI: 10.1007/s11033-010-0565-5]

6 **De Feo E**, Cefalo C, Arzani D, Amore R, Landolfi R, Grieco A, Ricciardi W, Miele L, Boccia S. A case-control study on the effects of the apolipoprotein E genotypes in nonalcoholic fatty liver disease. *Mol Biol Rep* 2012; **39**: 7381-7388 [PMID: 22350157 DOI: 10.1007/s11033-012-1570-7]

7 **Marrzoq LF**, Sharif FA, Abed AA. Relationship between ApoE gene polymorphism and coronary heart disease in Gaza Strip. *J Cardiovasc Dis Res* 2011; **2**: 29-35 [PMID: 21716749 DOI: 10.4103/0975-3583.78584]

8 **Salem NA**, Salem EA. Renoprotective effect of grape seed extract against oxidative stress induced by gentamicin and hypercholesterolemia in rats. *Ren Fail* 2011; **33**: 824-832 [PMID: 21787152 DOI: 10.3109/0886022X.2011.601832]

9 **Lee HS**. Mechanisms and consequences of hypertriglyceridemia and cellular lipid accumulation in chronic kidney disease and metabolic syndrome. *Histol Histopathol* 2011; **26**: 1599-1610 [PMID: 21972098 DOI:]

10 **Gheith O**, Sheashaa H, Abdelsalam M, Shoeir Z, Sobh M. Efficacy and safety of Monascus purpureus Went rice in children and young adults with secondary hyperlipidemia: a preliminary report. *Eur J Intern Med* 2009; **20**: e57-e61 [PMID: 19393480 DOI: 10.1016/j.ejim.2008.08.012]

11 **Lin CT**, Xu YF, Wu JY, Chan L. Immunoreactive apolipoprotein E is a widely distributed cellular protein. Immunohistochemical localization of apolipoprotein E in baboon tissues. *J Clin Invest* 1986; **78**: 947-958 [PMID: 3531238 DOI: 10.1172/JCI112685]

12 **Zhou TB**. Signaling pathways of apoE and its role of gene expression in glomerulus diseases. *J Recept Signal Transduct Res* 2013; **33**: 73-78 [PMID: 23384034 DOI: 10.3109/10799893.2013.765466]

13 **Chen G**, Paka L, Kako Y, Singhal P, Duan W, Pillarisetti S. A protective role for kidney apolipoprotein E. Regulation of mesangial cell proliferation and matrix expansion. *J Biol Chem* 2001; **276**: 49142-49147 [PMID: 11579084 DOI: 10.1074/jbc.M104879200]

14 **Zhang Y**, Yasumoto Y, Ikeda T, Takenouchi S, Sogabe A, Nosaki T, Che X, Zheng C, Haraguchi M, Akiyama S, Tsubouchi H. Apolipoprotein E regulates primary cultured human mesangial cell proliferation. *Nephron Exp Nephrol* 2006; **102**: e62-e70 [PMID: 16179831 DOI: 10.1159/000088402]

15 **Calandra S**, Gherardi E, Fainaru M, Guaitani A, Bartosek I. Secretion of lipoproteins, apolipoprotein A-I and apolipoprotein E by isolated and perfused liver of rat with experimental nephrotic syndrome. *Biochim Biophys Acta* 1981; **665**: 331-338 [PMID: 6793079 DOI: 10.1016/0005-2760(81)90018-7]

16 **Deighan CJ**, Caslake MJ, McConnell M, Boulton-Jones

JM, Packard CJ. Patients with nephrotic-range proteinuria have apolipoprotein C and E deficient VLDL1. *Kidney Int* 2000; **58**: 1238-1246 [PMID: 10972686 DOI: 10.1046/j.1523-1755.2000.00278.x]
17 **Russi G**, Furci L, Leonelli M, Magistroni R, Romano N, Rivasi P, Albertazzi A. Lipoprotein glomerulopathy treated with LDL-apheresis (Heparin-induced Extracorporeal Lipoprotein Precipitation system): a case report. *J Med Case Rep* 2009; **3**: 9311 [PMID: 20062740 DOI: 10.1186/1752-1947-3-9311]
18 **Zhou TB**, Qin YH, Lei FY, Su LN, Zhao YJ, Huang WF. Less gelatinases is associated with apolipoprotein E accumulation in glomerulosclerosis rats. *Histol Histopathol* 2012; **27**: 249-256 [PMID: 22207560]
19 **Zhou TB**, Qin YH, Ou C, Lei FY, Su LN, Huang WF, Zhao YJ. All-trans retinoic acid can regulate the expressions of gelatinases and apolipoprotein E in glomerulosclerosis rats. *Vascul Pharmacol* 2011; **55**: 169-177 [PMID: 21907828 DOI: 10.1016/j.vph.2011.08.223]
20 **Zhou TB**, Qin YH, Xu HL. Association of apoE gene expression and its gene polymorphism with nephrotic syndrome susceptibility: a meta-analysis of experimental and human studies. *Mol Biol Rep* 2012; **39**: 9347-9354 [PMID: 22760259 DOI: 10.1007/s11033-012-1751-4]
21 **Hayakawa M**, Okubo M, Katori H, Nagahama K, Murase T, Kobayashi T, Tanaka S, Nakanishi K, Odawara M, Matsushita H, Yamada A. A patient with apolipoprotein E2 variant (Q187E) without lipoprotein glomerulopathy. *Am J Kidney Dis* 2002; **39**: E15 [PMID: 11877595 DOI: 10.1053/ajkd.2002.31426]
22 **Luo B**, Huang F, Liu Q, Li X, Chen W, Zhou SF, Yu X. Identification of apolipoprotein E Guangzhou (arginine 150 proline), a new variant associated with lipoprotein glomerulopathy. *Am J Nephrol* 2008; **28**: 347-353 [PMID: 18046082 DOI: 10.1159/000111828]
23 **Zhou TB**, Liu YG, Lin N, Qin YH, Huang K, Shao MB, Peng DD. Relationship between angiotensin-converting enzyme insertion/deletion gene polymorphism and systemic lupus erythematosus/lupus nephritis: a systematic review and metaanalysis. *J Rheumatol* 2012; **39**: 686-693 [PMID: 22337243 DOI: 10.3899/jrheum.110863]
24 **Begg CB**, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**: 1088-1101 [PMID: 7786990]
25 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]
26 **Feussner G**, Wey S, Bommer J, Deppermann D, Grützmacher P, Ziegler R. Apolipoprotein E phenotypes and hyperlipidemia in patients under maintenance hemodialysis. *Hum Genet* 1992; **88**: 307-312 [PMID: 1733833]
27 **Hu P**, Qin YH, Lu L, Hu B, Jing CX, Lei FY, Li MF. Genetic variation of apolipoprotein E does not contribute to the lipid abnormalities secondary to childhood minimal change nephrotic syndrome. *Int Urol Nephrol* 2010; **42**: 453-460 [PMID: 19241136 DOI: 10.1007/s11255-009-9531-3]
28 **Oda H**, Yorioka N, Ueda C, Nishida Y, Yamakido M. Apolipoprotein E phenotype and renal disease. *Contrib Nephrol* 1997; **120**: 22-29 [PMID: 9257044]
29 **Eggertsen G**, Heimbürger O, Stenvinkel P, Berglund L. Influence of variation at the apolipoprotein E locus on lipid and lipoprotein levels in CAPD patients. *Nephrol Dial Transplant* 1997; **12**: 141-144 [PMID: 9027789 DOI: 10.1093/ndt/12.1.141]
30 **Werle E**, Fiehn W, Hasslacher C. Apolipoprotein E polymorphism and renal function in German type 1 and type 2 diabetic patients. *Diabetes Care* 1998; **21**: 994-998 [PMID: 9614620]
31 **Imura T**, Kimura H, Gejyo F. Apolipoprotein E phenotypes in hemodialysis patients. *Kidney Int Suppl* 1999; **71**: S245-S247 [PMID: 10412789]
32 **Güz G**, Nurhan Ozdemir F, Sezer S, Işiklar I, Arat Z, Turan M, Haberal M. Effect of apolipoprotein E polymorphism on serum lipid, lipoproteins, and atherosclerosis in hemodialysis patients. *Am J Kidney Dis* 2000; **36**: 826-836 [PMID: 11007687 DOI: 10.1053/ajkd.2000.17682]
33 **Erdogan M**, Eroglu Z, Biray C, Karadeniz M, Cetinkalp S, Kosova B, Gunduz C, Topcuoglu N, Ozgen G, Yilmaz C. The relationship of the apolipoprotein E gene polymorphism Turkish Type 2 diabetic patients with and without nephropathy. *J Endocrinol Invest* 2009; **32**: 219-222 [PMID: 19542737]
34 **Zahálková J**, Vaverková H, Novotný D, Kosatíková Z. Impaired triglyceride tolerance in hemodialysis patients with different apolipoprotein E (apo E) isoforms. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2002; **146**: 73-76 [PMID: 12572901]
35 **Arikan H**, Koc M, Sari H, Tuglular S, Ozener C, Akoglu E. Associations between apolipoprotein E gene polymorphism and plasminogen activator inhibitor-1 and atherogenic lipid profile in dialysis patients. *Ren Fail* 2007; **29**: 713-719 [PMID: 17763167 DOI: 10.1080/08860220701460129]
36 **Joss N**, Jardine A, Gaffney D, Boulton-Jones JM. Influence of apolipoprotein E genotype on progression of diabetic nephropathy. *Nephron Exp Nephrol* 2005; **101**: e127-e133 [PMID: 16113584 DOI: 10.1159/000087577]
37 **Ma SW**, Benzie IF, Yeung VT. Type 2 diabetes mellitus and its renal complications in relation to apolipoprotein E gene polymorphism. *Transl Res* 2008; **152**: 134-142 [PMID: 18774543 DOI: 10.1016/j.trsl.2008.07.003]
38 **Lim PS**, Liu CS, Hong CJ, Wei YH. Prevalence of apolipoprotein E genotypes in ischaemic cerebrovascular disease in end-stage renal disease patients. *Nephrol Dial Transplant* 1997; **12**: 1916-1920 [PMID: 9306343 DOI: 10.1093/ndt/12.9.1916]
39 **Kimura H**, Suzuki Y, Gejyo F, Karasawa R, Miyazaki R, Suzuki S, Arakawa M. Apolipoprotein E4 reduces risk of diabetic nephropathy in patients with NIDDM. *Am J Kidney Dis* 1998; **31**: 666-673 [PMID: 9531184 DOI: 10.1053/ajkd.1998.v31.pm9531184]
40 **Lehtinen S**, Rantalaiho V, Wirta O, Pasternack A, Laippala P, Koivula T, Lehtimäki T. Apolipoprotein E gene polymorphism, hypercholesterolemia and glomerular filtration rate in type 2 diabetic subjects: a 9-year follow-up study. *J Biomed Sci* 2003; **10**: 260-265 [PMID: 12595762 DOI: 10.1159/000068712]
41 **Kwon MK**, Rhee SY, Chon S, Oh S, Woo JT, Kim SW, Kim JW, Kim YS, Jeong KH, Lee SH, Lee TW, Ihm CG. Association between apolipoprotein E genetic polymorphism and the development of diabetic nephropathy in type 2 diabetic patients. *Diabetes Res Clin Pract* 2007; **77** Suppl 1: S228-S232 [PMID: 17624465 DOI: 10.1016/j.diabres.2007.03.009]
42 **Kahraman S**, Kiykim AA, Altun B, Gençtoy G, Arici M, Gulsun M, Erdem Y, Yasavul U, Turgan C, Cağlar S. Apolipoprotein E gene polymorphism in renal transplant recipients: effects on lipid metabolism, atherosclerosis and allograft function. *Clin Transplant* 2004; **18**: 288-294 [PMID: 15142050 DOI: 10.1111/j.1399-0012.2004.00162.x]
43 **Xiang G**, Xia B, He Y. [The relationship of Apo E2 and renal insufficiency lipid levels in NIDDM]. *Zhonghua Yixue Zazhi* 1999; **79**: 339-341 [PMID: 11715472]
44 **Li HF**, Han CF, Wang YX, Lu YS, Zou HQ, Xu QQ. Effect of apolipoprotein E gene polymorphism on serum lipid level before and after renal transplantation. *Transplant Proc* 2010; **42**: 2513-2517 [PMID: 20832534 DOI: 10.1016/j.transproceed.2010.04.023]
45 **Maluf DG**, Mas VR, Archer KJ, Yanek K, King A, Ferreira-Gonzalez A, Fisher RA, Posner M. Apolipoprotein E genotypes as predictors of high-risk groups for developing hyperlipidemia in kidney transplant recipients undergoing sirolimus treatment. *Transplantation* 2005; **80**: 1705-1711 [PMID: 16378065 DOI: 10.1097/01.tp.0000187869.04180.7f]
46 **Leiva E**, Mujica V, Elematore I, Orrego R, Díaz G, Prieto M, Arredondo M. Relationship between Apolipoprotein E poly-

morphism and nephropathy in type-2 diabetic patients. *Diabetes Res Clin Pract* 2007; **78**: 196-201 [PMID: 17481771 DOI: 10.1016/j.diabres.2007.03.018]

47 **Stoycheff N**, Stevens LA, Schmid CH, Tighiouart H, Lewis J, Atkins RC, Levey AS. Nephrotic syndrome in diabetic kidney disease: an evaluation and update of the definition. *Am J Kidney Dis* 2009; **54**: 840-849 [PMID: 19556043 DOI: 10.1053/j.ajkd.2009.04.016]

48 **Zhou TB**, Qin YH, Lei FY, Su LN, Zhao YJ, Huang WF. All-trans retinoic acid regulates the expression of apolipoprotein E in rats with glomerulosclerosis induced by Adriamycin. *Exp Mol Pathol* 2011; **90**: 287-294 [PMID: 21385580 DOI: 10.1016/j.yexmp.2011.03.001]

49 **Makówka A**, Dryja P, Chwatko G, Bald E, Nowicki M. Treatment of chronic hemodialysis patients with low-dose fenofibrate effectively reduces plasma lipids and affects plasma redox status. *Lipids Health Dis* 2012; **11**: 47 [PMID: 22564753 DOI: 10.1186/1476-511X-11-47]

50 **Toms TE**, Smith JP, Panoulas VF, Blackmore H, Douglas KM, Kitas GD. Apolipoprotein E gene polymorphisms are strong predictors of inflammation and dyslipidemia in rheumatoid arthritis. *J Rheumatol* 2012; **39**: 218-225 [PMID: 22174202 DOI: 10.3899/jrheum.110683]

51 **Alvim RO**, Freitas SR, Ferreira NE, Santos PC, Cunha RS, Mill JG, Krieger JE, Pereira AC. APOE polymorphism is associated with lipid profile, but not with arterial stiffness in the general population. *Lipids Health Dis* 2010; **9**: 128 [PMID: 21059196 DOI: 10.1186/1476-511X-9-128]

52 **Rahimi Z**, Vaisi-Raygani A, Pourmotabbed T. Association between apolipoprotein ε4 allele, factor V Leiden, and plasma lipid and lipoprotein levels with sickle cell disease in Southern Iran. *Mol Biol Rep* 2011; **38**: 703-710 [PMID: 20373031 DOI: 10.1007/s11033-010-0157-4]

53 **Smart MC**, Dedoussis G, Louizou E, Yannakoulia M, Drenos F, Papoutsakis C, Maniatis N, Humphries SE, Talmud PJ. APOE, CETP and LPL genes show strong association with lipid levels in Greek children. *Nutr Metab Cardiovasc Dis* 2010; **20**: 26-33 [PMID: 19403283 DOI: 10.1016/j.numecd.2009.02.005]

54 **Jofre-Monseny L**, Minihane AM, Rimbach G. Impact of apoE genotype on oxidative stress, inflammation and disease risk. *Mol Nutr Food Res* 2008; **52**: 131-145 [PMID: 18203129 DOI: 10.1002/mnfr.200700322]

55 **Arora S**, Husain M, Kumar D, Patni H, Pathak S, Mehrotra D, Reddy VK, Reddy LR, Salhan D, Yadav A, Mathieson PW, Saleem MA, Chander PN, Singhal PC. Human immunodeficiency virus downregulates podocyte apoE expression. *Am J Physiol Renal Physiol* 2009; **297**: F653-F661 [PMID: 19553347 DOI: 10.1152/ajprenal.90668.2008]

56 **Mahley RW**. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988; **240**: 622-630 [PMID: 3283935 DOI: 10.1126/science.3283935]

57 **Greenow K**, Pearce NJ, Ramji DP. The key role of apolipoprotein E in atherosclerosis. *J Mol Med* (Berl) 2005; **83**: 329-342 [PMID: 15827760 DOI: 10.1007/s00109-004-0631-3]

58 **Anthopoulos PG**, Hamodrakas SJ, Bagos PG. Apolipoprotein E polymorphisms and type 2 diabetes: a meta-analysis of 30 studies including 5423 cases and 8197 controls. *Mol Genet Metab* 2010; **100**: 283-291 [PMID: 20381392 DOI: 10.1016/j.ymgme.2010.03.008]

59 **Ukkola O**, Kunnari A, Jokela M, Päivänsalo M, Kesäniemi YA. ApoE phenotype is associated with inflammatory markers in middle-aged subjects. *Inflamm Res* 2009; **58**: 54-59 [PMID: 19115037 DOI: 10.1007/s00011-008-8215-2]

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META-ANALYSIS

Accuracy of early detection of colorectal tumours using stool methylation markers: A meta-analysis

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Abstract

AIM: To evaluate the accuracy of methylation of genes in stool samples for diagnosing colorectal tumours.

METHODS: Electronic databases including PubMed, Web of Science, Chinese Journal Full Text Database and Wanfang Journals Full-text Database were searched to find relevant original articles about methylated genes used in diagnosing colorectal tumours. Quality assessment of diagnostic accuracy studies items were used to evaluate the quality of the included articles, and the Meta-disc 1.4 and SPSS 13.0 software programs were used for data analysis.

RESULTS: Thirty-four articles met the inclusion criteria, and 4151 patients were included. Pooled diagnostic performances of SFRP2 methylation for colorectal cancer (CRC) provided the following results: the sensitivity was 79% (95%CI: 75%-82%), the specificity was 93% (95%CI: 90%-96%), the diagnostic odds ratio (DOR) was 47.57 (95%CI: 20.08-112.72), and the area under the curve was 0.9565. Additionally, the results of accuracy of SFRP2 methylation for detecting colorectal adenomas were as follows: the sensitivity was 43% (95%CI: 38%-49%), the specificity was 94% (95%CI: 91%-97%), the DOR was 11.06 (95%CI: 5.77-21.18), and the area under the curve was 0.9563.

CONCLUSION: Stool-based DNA testing may be useful for non-invasively diagnosing colorectal tumours, and SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis.

Key words: Colorectal carcinoma; Colorectal adenoma; Stool; Methylation; Meta-analysis

Core tip: The analysis of stool methylation markers as a non-invasive test is important for the early diagnosis of colorectal tumours. However, no consensus has been reached with regard to the role of stool methylation markers in colorectal tumour diagnosis. We performed a meta-analysis of 34 articles, and the pooled results showed that stool methylation markers could be used as a valuable diagnostic and predictive tool for colorectal tumours and SFRP2 methylation serves as a promising marker with great potential in early colorectal cancer diagnosis.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer-related deaths in Western countries[1,2]. The 5-year survival of stage I CRC has reached 90%[3], but less than 10% of CRC cases have distant metastases[4]. However, most cases of CRC are diagnosed at the middle or late stage because no typical symptoms for early-stage CRC exist[5]. Therefore, the diagnosis of CRC at early stages has great importance for reducing CRC mortality.

Early diagnosis of CRC will help reduce mortality and the costs for surgery. The current colonoscopy screening test is of high efficacy, but the acceptability of this procedure in the general public is rather low. As an available non-invasive method, faecal testing has a unique advantage when compared to other screening modalities. Although faecal occult blood testing (FOBT) has been confirmed to reduce mortality due to CRC, the test has little or no impact on the incidence of CRC because of its low-level sensitivity for the detection of adenoma[6], *i.e.*, a sensitivity of only 10%-20%[7]. Compared to FOBT, the most important advantage of methylation markers in stool samples is their higher accuracy and sensitivity for the diagnosis of premalignant lesions of CRC[8].

DNA methylation often occurs in the early stage of CRC, and many studies have been performed on the diagnosis of colorectal tumours by determining the methylation of genes in stool samples. However, the results of these studies are variable although inspiring. Thus, this meta-analysis was conducted to assess the accuracy of the detection of colorectal tumours using methylation markers in stool samples.

MATERIALS AND METHODS

Search strategy

A literature search was performed independently by two investigators (Zhang H and Qi J) using the following databases: PubMed, Web of Science, Chinese Journal Full Text Database and Wanfang Journals Full-text Database. All references that were cited in these studies and all published reviews were also searched. All English and Chinese references for analyses were published before January 2013. The following keywords were used in the search strategy: "colon/rectal/colorectal", "cancer/tumours", "stool," and "methylation". In this meta-analysis, 2 × 2 tables were constructed from each study for the true-positive, false-negative, true-negative and false-positive values.

Inclusion and exclusion criteria

Eligible studies were required to meet all of the following criteria: (1) the data were independent; (2) CRC was diagnosed by analyzing DNA methylation in stool sample; (3) patients were diagnosed with colorectal cancer or colorectal adenomas by pathology; and (4) the colonoscopy result of control individuals was normal.

Exclusion criteria for this meta-analysis were as follows: (1) studies on secondary CRC or primary CRC with distant metastases and (2) studies on CRC patients receiving chemotherapy or curative surgery.

Data extraction and quality assessment

The following data were extracted from each study: author, year of publication, country or region, sample size, the name of genes, the detection method of methylation and the study design. The data were independently extracted by two investigators (Zhang H and Qi J), and discrepancies were solved by a third investigator (Zhu YQ) and collective discussion. Quality Assessment of studies of Diagnostic Accuracy[9] (QUADAS) was used to assess the quality of the primary studies with diagnostic accuracy, and quality scoring was appraised based on the empirical evidence, the experts' opinions and the formal consensus. Score of 1, 0 or -1 was given to the articles that were in compliance with the standards completely, unclear or out of standards, respectively, and the full score was 14.

Statistical analysis

All statistics were calculated and then combined using a random-effects model and 95%CI as effect measurements. The diagnostic odds ratio (DOR) reflects the relationship between the result of the diagnostic test and the disease. The summary receiver operation characteristic (SROC) curve displays the trade-off between sensitivity and specificity and represents a global summary of test performance. We used the Q-value, which is the intersection point of the SROC curve with a diagonal line from the left upper corner to the right lower corner of the receiver operation characteristic (ROC) space, which corresponds to the highest value of sensitivity and specificity for the test. The positive likelihood ratio (PLR) represents the value by which the odds of the disease increase when a test is positive, whereas the negative likelihood ratio (NLR) shows the value by which the odds of the disease decrease when a test is negative. Statistical heterogeneity was assessed using the Chi-square test, and alpha significance testing was performed at the two-tailed 0.05 level. The professional statistical software programs (Meta-DiSc 1.4 and SPSS 13.0) were used for analysis. Publication bias was assessed by Egger analysis.

RESULTS

The literature search retrieved 453 citations, of which 344 were excluded because they were duplicates. Of the 109 potentially eligible studies, 75 publications were excluded because they did not investigate colorectal tumour studies (n = 6), included no diagnostic value studies (n = 15), were reviews (n = 26) or had overlapping data (n = 28). Finally, 34 studies that focused on the target patient spectrum were included (Figure 1).

Study characteristics

Of the 34 studies, 7 were Chinese and 27 were English,

Table 1 The characteristics of the included studies in the meta-analysis and QUADAS scores

Ref.	Country/ region	Methylated genes	N	CRC		Adenoma		Normal		Blind design	Detection method	QUADAS score
				+	-	+	-	+	-			
Ahlquist *et al*[10]	Ireland	*Vimentin/NDRG4/BMP3/TFPI2*	98	26	4	18	4	5	41	Yes	QuARTS	11
Bosch *et al*[11], 2011	Netherlands	*PHACTR3*	185	40	25	6	13	4	97	Unclear	qMSP	10
		GATA4	160	29	11	3	16	6	95			
		OSMR	185	25	40	4	15	7	94			
Ahlquist *et al*[12]	Ireland	*PHACTR3*	639	214	38	51	43	29	264	Yes	QuARTS	11
Azuara *et al*[13]	Spain	*RARB2/P16/MGMT/APC*	98	25	13	20	20	0	20	Yes	MS-MCA	10
		RARB2	85	11	23	7	31	0	13			
		P16	77	9	21	6	28	0	13			
		MGMT	80	9	19	3	34	0	15			
		APC	77	9	19	9	25	0	15			
Tang *et al*[14]	China	*SRRP2*	262	142	27	29	34	2	28	Yes	MSP	9
Baek *et al*[15]	South Korea	*Vimentin/MGMT/MLH1*	149	45	15	31	21	5	32	Yes	MSP	9
		MLH1	149	18	42	6	46	0	37			
		Vimentin	149	23	37	8	44	0	37			
		MGMT	149	31	29	19	33	5	32			
Li *et al*[16], 2009	United States	*Vimentin*	80	9	13	9	11	2	36	Unclear	Methl-BEAMing	5
Melotte *et al*[17], 2009	Netherlands	*NDRG4*	150	42	33	nr	nr	3	72	Yes	qMSP	11
Ausch *et al*[18], 2009	United States	*IGTA4*	37	nr	nr	7	2	6	22	Unclear	qMSP	4
Hellebrekers *et al*[19], 2009	Netherlands	*GATA4*	150	44	31	nr	nr	9	66	Yes	qMSP	10
Mayor *et al*[20], 2009	Spain	*EN1*	60	8	22	nr	nr	1	29	Unclear	MS-MCA	7
Kim *et al*[21], 2009	United States	*OSMR/SFRP1*	42	12	8	6	11	0	5	Yes	qMSP	9
		OSMR	201	35	54	2	14	4	92			
		SFRP1	52	11	9	5	12	0	15			
Nagasaka *et al*[22], 2009	Japan	*SFRP2*	253	53	31	18	38	9	104	Unclear	COBRA	10
		RASSF2	253	38	46	7	49	6	107			
Glöckner *et al*[23], 2009	United States	*TFPI2*	129	44	14	7	19	2	43	Yes	qMSP	12
Wang *et al*[24], 2008	China	*SFRP2*	133	60	9	21	13	2	28	Yes	MethyLight	8
Oberwalder *et al*[25], 2008	Australia	*SFRP2*	19	nr	nr	6	7	0	6	Yes	MethyLight	9
Itzkowitz *et al*[26], 2008	United States	*Vimentin*	80	9	13	9	11	2	36	Yes	MSP	13
Huang *et al*[27], 2007	China	*SFRP2/HPP1/MGMT*	97	50	2	15	6	1	23	Yes	MSP	8
		SFRP2	97	49	3	11	10	1	23			
		HPP1	97	37	15	12	9	0	24			
		MGMT	97	25	27	6	15	0	24			
Itzkowitz *et al*[28], 2007	United States	*Vimentin/HLTF*	162	31	9	nr	nr	19	103	Yes	MSP	13
		HLTF	162	15	25	nr	nr	9	113			
		Vimentin	162	29	11	nr	nr	16	106			
Abbaszadegan *et al*[29], 2007	Hong Kong	*p16*	45	5	20	nr	nr	0	20	Unclear	MSP	8
Zhang *et al*[30], 2007	Germany	*SFRP1*	44	16	4	7	0	2	15	Yes	MSP	9
Leung *et al*[31], 2007	Hong Kong	*SFRP2/MGMT/MLH1/HLTF/ ATM/APC*	75	16	4	18	7	3	27	Yes	MSP	13
		SFRP2	75	6	14	3	22	2	28			
		MGMT	75	4	16	3	22	0	30			
		MLH1	75	4	16	3	22	0	30			
		HLTF	75	5	15	5	20	1	29			
		ATM	75	5	15	5	20	0	30			
		APC	75	4	16	4	21	0	30			
Petko *et al*[32], 2005	United States	*MGMT/ CDKN2A/ MLH1*	48	nr	nr	16	13	7	12	Yes	MSP	9
		CDKN2A	48	nr	nr	9	20	3	16			
		MGMT	48	nr	nr	14	15	5	14			
		MLH1	48	nr	nr	0	29	2	17			
Lenhard *et al*[33], 2005	Germany	*HIC1*	71	11	15	4	9	0	32	Yes	MSP	11
Chen *et al*[34], 2005	United States	*Vimentin*	263	43	51	6	44	8	111	Yes	MSP	11
Müller *et al*[35], 2004	Australia	*SFRP2/ SRRP5*	39	20	3	nr	nr	8	8	Unclear	MethyLight	5
		SRRP2	39	19	4	nr	nr	4	12			
		SRRP5	39	18	5	nr	nr	5	11			
Xu *et al*[36], 2012	China	*SFRP2*	90	20	10	15	15	1	29	Unclear	MSP	5
Kang *et al*[37], 2011	China	*MGMT/ MAL/ CDKN2A*	119	64	5	17	7	2	24	Unclear	MSP	7
		MAL	119	54	15	14	10	1	25			
		CDKN2A	119	36	33	10	14	0	26			
		MGMT	119	38	31	9	15	1	25			
Zhang *et al*[38], 2011	China	*Vimentin/ OSMR/ TFPI2*	107	52	8	13	4	4	26	Unclear	MSP	9
		Vimentin	107	32	28	5	12	0	30			
		OSMR	107	41	19	7	10	0	30			
		TFPI2	107	45	15	11	6	4	26			

Fu *et al*[39], 2010	China	*Vimentin*	22	5	9	nr	nr	0	8	Unclear	MSP	5
Ling *et al*[40], 2009	China	*P16*	108	47	14	16	11	1	19	Unclear	MSP	7
Cheng *et al*[41], 2007	China	*SFRP2*	97	49	3	11	10	1	23	Unclear	MSP	5
Zhao *et al*[42], 2009	China	*NDRG4*	114	64	20	nr	nr	3	27	Unclear	MSP	6
Chang *et al*[43], 2010	South Korea	*IGTA4/SFRP2/P16*	86	21	9	18	7	1	30	Yes	MSP	8
		IGTA4	86	11	19	4	21	0	31			
		SFRP2	86	18	12	11	14	0	31			
		P16	86	12	18	6	19	1	30			

+: Represents the number of individuals with the disease when the DNA methylation test was positive; -: Represents the number of individuals with the disease when the DNA methylation test was negative; nr: Not reported; N: Total number. CRC: Colorectal cancer.

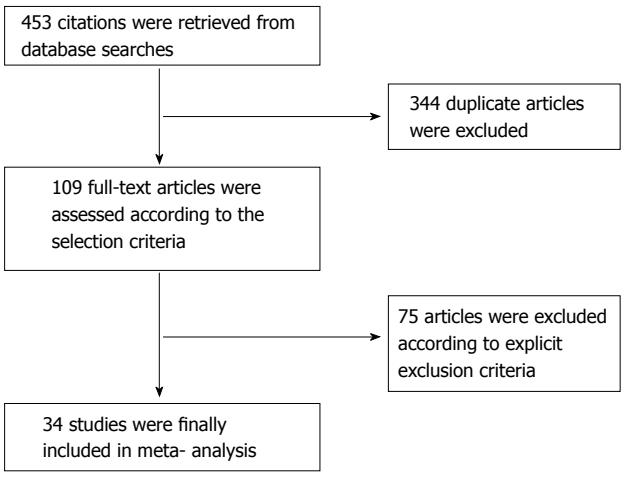

Figure 1 Flowchart of the study selection.

and they included 4151 patients (Table 1). These studies were performed in 10 countries or regions (including China, the United States, the Netherlands, Spain, Japan, Germany, Iran, Hong Kong, Austria and South Korea). In these studies, 31 evaluated CRC, and 26 evaluated colorectal adenoma. Twenty-two studies focused on the methylation of a single gene, and the other 12 studies involved the methylation of multiple genes.

Genes evaluated in these studies are mainly involved in three types of regulation pathways: the Wnt pathway, the DNA damage repair pathway and other pathways. Four genes of the Wnt pathway were involved in 10 studies: secreted frizzled-related proteins (*SFRP1*, *SFRP2*, *SFRP5*) and adenomatous polyposis coli (*APC*). Two genes of the DNA damage repair pathway were involved in 7 studies: O-6-methylguanine-DNA methyltransferase (MGMT) and Mut L homologue 1 (*MLH1*). Twenty-seven studies involved 16 genes of other pathways: vimentin, oncostain M receptor-β (*OSMR*), phosphatase and actin regulator 3 (*PHACTR3*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*), tissue factor pathway inhibitor 2 (*TFPI2*), hyperplastic polyposis protein gene (*HPP1*), GATA4, human lactoferrin (*HLTF*), *ATM*, ras association domain family 2 (*RASSF2*), *RARB2*, hypermethylated in cancer 1 (*HIC*), engrailed gene (*EN1*), N-myc downstream-regulated gene family (*NDRG4*), *IGTA4* and T-cell differentiation protein (*MAL*).

Qualitative and quantitative methods were the two main types of methods used for methylation detection. The qualitative method included methylation-specific PCR (MSP) and methylation-specific melting curve analysis (MS-MCA). The quantitative method included Methl-BEAMing; quantitative MSP (qMSP); MethyLight; combined bisulfite restriction analysis (COBRA); and quantitative, allele-specific, real-time target and signal amplification (QuARTS).

Colorectal carcinoma meta-analysis

The colorectal carcinoma results were pooled from 31 studies and are shown in Table 2. The meta-analysis showed that the sensitivity and specificity of gene methylation for the detection of colorectal carcinoma were 73% (95%CI: 71%-75%) and 92% (95%CI: 90%-93%), respectively. The PLR was 7.94 (95%CI: 6.08-10.36), the NLR was 0.31 (95%CI: 0.25-0.39), the DOR was 30.86 (95%CI: 22.33-42.66), and the symmetric area under the curve was 0.9286.

Heterogeneity was significant for the sensitivity ($P < 0.001$), specificity ($P = 0.0012$), PLR ($P = 0.0023$), NLR ($P < 0.001$), and DOR ($P = 0.0245$).

Of the involved regulation mechanisms, we found that DOR and AUC of the methylated genes belonging to the Wnt pathway were higher than the genes of the DNA damage repair pathway and other pathways. The sensitivity, specificity, DOR and AUC of different methylated genes in the three types of pathways were calculated (Table 2), and the results indicated that the accuracy of faecal SFRP2 methylation in the diagnosis of colorectal carcinoma was higher than that of other genes, with a sensitivity of 79% (95%CI: 75%-82%) (Figure 2A), a specificity of 93% (95%CI: 90%-96%) (Figure 2B), a DOR of 47.57 (95%CI: 20.08-112.72), and the area under the curve of 0.9565 (Figure 2C).

Colorectal adenoma meta-analysis

Pooled colorectal adenoma analysis (Table 3), including 26 studies, provided the following results: the sensitivity and specificity of gene methylation for colorectal adenoma diagnosis were 51% (95%CI: 47%-54%) and 92% (95%CI: 90%-93%), respectively. The PLR was 5.52 (95%CI: 4.23-7.19), the NLR was 0.52 (95%CI: 0.44-0.61), and the DOR and symmetric area under the curve were 12.61 (95%CI: 8.66-18.37) and 0.8830, respectively.

Heterogeneity was also significant regarding sensitivity ($P < 0.001$), specificity ($P = 0.0233$), PLR ($P = 0.1166$), NLR ($P < 0.001$), and DOR ($P = 0.0565$).

Table 2 Methylation of pooled genes for the diagnosis of colorectal cancer

Wnt pathway	DNA damage repair pathway	Other pathways	SE (95%CI)	SP (95%CI)	DOR (95%CI)	AUC
Wnt pathway	DNA damage repair pathway	Other pathways	73% (0.71-0.75)	92% (90%-93%)	30.86 (22.33-42.66)	0.929
Wnt pathway	-	-	74% (70%-77%)	93% (90%-95%)	33.92 (17.73-64.90)	0.932
-	DNA damage repair pathway	-	42% (36%-47%)	97% (94%-99%)	12.87 (5.98-27.72)	0.730
-	-	Other pathways	57% (55%-60%)	94% (93%-95%)	20.93 (15.56-28.15)	0.921
SFRP2	-	-	79% (75%-82%)	93% (90%-96%)	47.57 (20.08-112.72)	0.957
-	MGMT	-	47% (40%-53%)	95% (90%-98%)	11.67 (5.10-26.67)	0.709
-	MLH	-	28% (18%-39%)	100% (95%-100%)	23.68 (3.02-185.44)	0.500
-	-	Vimentin	48% (42%-54%)	93% (90%-95%)	14.95 (8.99-24.84)	0.862
-	-	OSMR	47% (40%-54%)	95% (91%-98%)	14.66 (5.06-42.47)	0.225
-	-	P16	50% (42%-58%)	98% (92%-100%)	24.39 (7.26-81.96)	0.975
SFRP2	MGMT	-	69% (66%-72%)	94% (91%-96%)	33.24 (16.76-65.93)	0.946
SFRP2	MLH	-	72% (68%-75%)	94% (92%-96%)	43.03 (20.15-91.87)	0.953
SFRP2	MLH	Vimentin	64% (61%-67%)	94% (92%-95%)	27.11 (16.48-44.61)	0.934
SFRP2	MLH	OSMR	65% (62%-69%)	95% (93%-96%)	33.10 (17.12-63.98)	0.951
SFRP2	MLH	P16	68% (64%-71%)	95% (93%-97%)	38.86 (20.11-67.54)	0.952

SE: Sensitivity; SP: Specificity; DOR: Diagnostic odds ratio; AUC: The area under the curve.

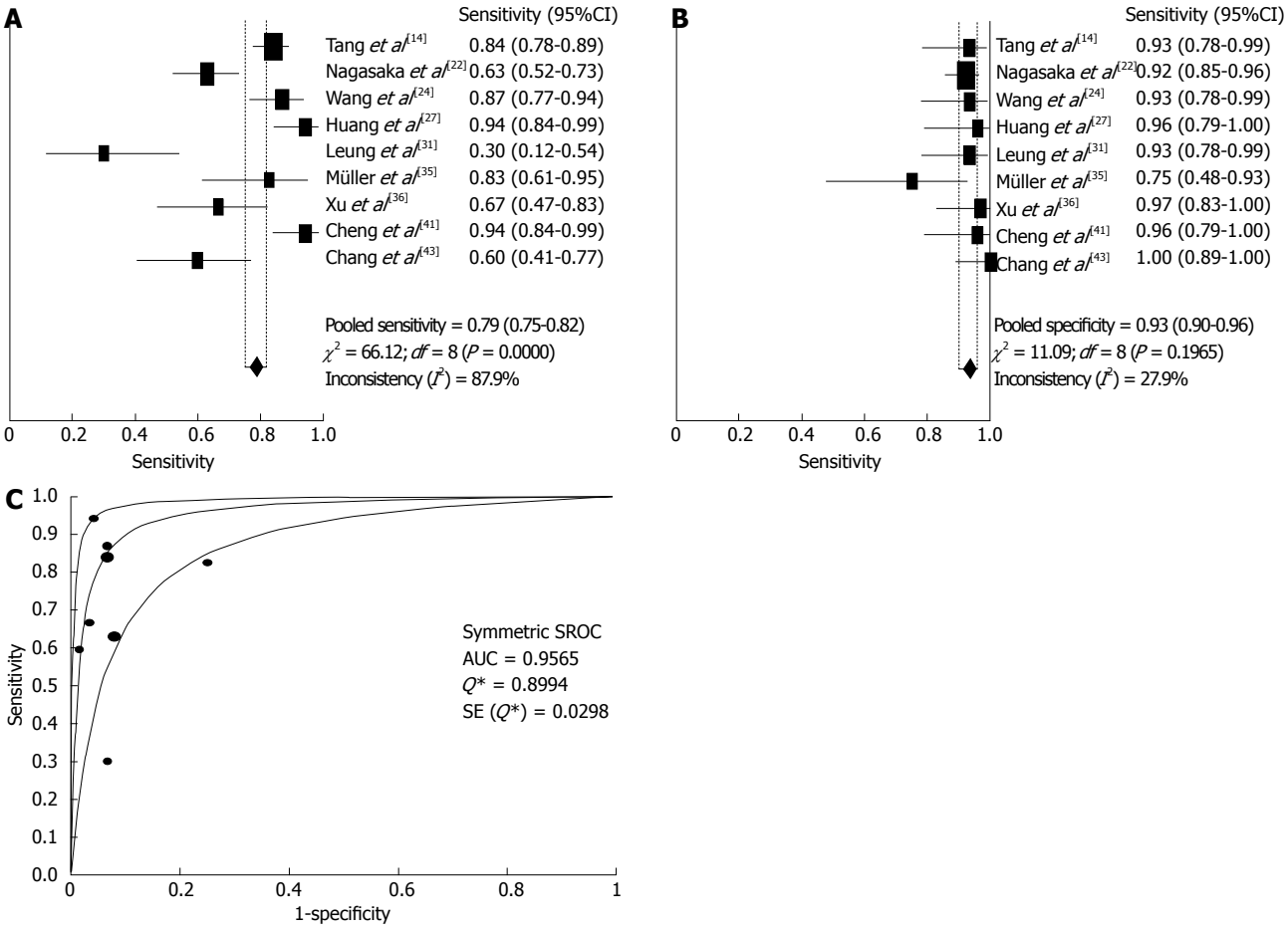

Figure 2 Forest plot of SFRP2 methylation in the diagnosis of colorectal cancer. A: The sensitivity of SFRP2 methylation in stool samples used for colorectal carcinoma diagnosis. The point estimates of specificity from each study are shown as red squares; B: The specificity of SFRP2 methylation in stool samples used for colorectal cancer diagnosis. The point estimates of specificity from each study are shown as blue squares; C: The summary receiver operating characteristic curves of SFRP2 methylation assays used for diagnosis of colorectal carcinoma. Red circles represent each study that was included in the meta-analysis. The size of each study is indicated by the size of the red circle. Summary receiver operating characteristic curves summarize the overall diagnostic accuracy. Error bars indicate the 95%CI, and df indicates the degrees of freedom.

The DOR and AUC of the methylated Wnt pathway genes were higher than those of the genes of the DNA damage repair pathway and other pathways when grouping all of the genes by pathway for analysis. In these

Table 3 Methylation of pooled genes for the diagnosis of colorectal adenomas

Wnt pathway	DNA damage repair pathway	Other pathways	SE (95%CI)	SP (95%CI)	DOR (95%CI)	AUC
Wnt pathway	DNA damage repair pathway	Other pathways	51% (47%-54%)	92% (90%-93%)	12.61 (8.66-18.37)	0.883
Wnt pathway	-	-	40% (35%-46%)	95% (92%-97%)	10.81 (6.43-18.16)	0.932
-	DNA damage repair pathway	-	21% (17%-27%)	95% (91%-97%)	4.23 (2.01-8.88)	0.672
-	-	Other pathways	32% (28%-35%)	94% (93%-95%)	7.78 (5.48-11.05)	0.873
SFRP2	-	-	43% (38%-49%)	94% (91%-97%)	11.06 (5.77-21.18)	0.956
-	MGMT	-	29% (22%-36%)	93% (87%-96%)	4.42 (2.18-8.95)	0.614
-	MLH	-	8% (4%-16%)	98% (92%-100%)	2.35 (0.14-40.83)	-
-	-	Vimentin	23% (17%-31%)	95% (92%-98%)	8.30 (2.60-26.55)	0.898
-	-	OSMR	25% (14%-39%)	95% (91%-98%)	5.20 (1.44-18.82)	0.817
-	-	P16	33% (23%-44%)	97% (89%-100%)	13.27 (3.40-51.83)	0.97
SFRP2	MLH	-	34% (29%-39%)	95% (92%-97%)	9.62 (4.64-19.93)	0.947
SFRP2	MGMT	-	38% (33%-42%)	94% (91%-96%)	7.85 (4.79-12.87)	0.753
SFRP2	-	OSMR	41% (35%-46%)	95% (92%-96%)	9.25 (5.13-16.69)	0.948
SFRP2	-	Vimentin	36% (32%-41%)	95% (93%-96%)	9.88 (5.55-17.57)	0.946
SFRP2	-	P16	41% (36%-46%)	95% (92%-97%)	10.37 (6.21-17.31)	0.948
SFRP2	MGMT	Vimentin	34% (30%-38%)	94% (92%-96%)	7.81 (4.96-12.29)	0.804
SFRP2	MGMT	OSMR	36% (32%-41%)	94% (92%-96%)	7.25 (4.61-11.39)	0.775
SFRP2	MGMT	P16	37% (33%-41%)	94% (92%-96%)	7.92 (5.14-12.21)	0.772
SFRP2	MLH	Vimentin	31% (27%-35%)	95% (93%-97%)	8.99 (4.95-16.31)	0.944
SFRP2	MLH	OSMR	33% (29%-38%)	95% (93%-97%)	8.37 (4.50-15.59)	0.941
SFRP2	MLH	P16	34% (30%-38%)	95% (93%-97%)	9.98 (5.45-18.27)	0.947

SE: Sensitivity; SP: Specificity; DOR: Diagnostic odds ratio; AUC: The area under the curve.

regulation mechanisms, we also found that the sensitivity and specificity of the methylated genes in the Wnt pathway were higher than those in the DNA damage repair pathway and the other pathway. The sensitivity, specificity, DOR and AUC of the different methylated genes in the three types of pathways were calculated (Table 3), and the results indicated that the values of DOR and AUC of P16 and SFRP2 were higher than those of other genes, but the accuracy of faecal SFRP2 methylation for the diagnosis of colorectal adenoma was higher than that of P16 methylation according to sensitivity (Figure 3).

Meta-regression

In the meta-regression analysis, the difference in relative diagnostic odds ratio (RDOR) values between the higher and lower quality studies was not significant. We also noted that the differences between the blinded and non-blinded methods, qualitative and quantitative methods, single- and multiple-gene methylation did not reach statistical significance, indicating that these potential factors did not substantially affect the diagnostic accuracy, as shown in Table 4.

Publication bias

In our meta-analysis, publication bias was evaluated using the Egger test. The results showed no significant publication bias among the studies of SFRP2 methylation in faecal samples from CRC or adenoma patients (Figure 4).

DISCUSSION

It is widely accepted that DNA methylation in stool samples may be valuable for increasing the rate of CRC detection at earlier stage[44]. In the present study, we focused on the detection performance of gene methylation in stool samples for patients with colorectal tumours. Our analysis suggests that the specificity of SFRP2 methylation is high (93% for CRC and 94% for colorectal adenoma) for the detection of colorectal tumours; however, it has moderate (79%) and low sensitivity (43%) for diagnosing CRC and adenoma, respectively. Compared to FOBT, with a sensitivity of 14% for colorectal tumour diagnosis[45], the detection accuracy of faecal methylation biomarkers was higher as a CRC screening method.

The DOR is an indicator of test accuracy. The value of the DOR ranges from 0 to infinity, and higher values indicate better discriminatory test performance. In this meta-analysis, we found that the DOR of faecal SFRP2 methylation for colorectal carcinoma and adenoma were 47.57 and 11.06, respectively, which indicated a high level of overall accuracy for CRC and a low level for adenoma. The SROC curve represents an overall measure of the discriminatory power of a test. The area under the curve of 1 for any test indicates that the test is excellent. Our data showed that the area under the curve (AUC) of the SROC curve for faecal SFRP2 methylation for the diagnosis of colorectal carcinoma and adenoma were 0.9565 and 0.9563, respectively, which indicated that faecal SFRP2 methylation is an excellent diagnostic biomarker for colorectal tumours.

Because the DOR and SROC curve are not easy to use in clinical practice, the likelihood ratios are considered to be more clinically meaningful. For a high-quality diagnostic test, a PLR of > 10 or an NLR < 0.1 is typically required. However, our meta-analysis showed that neither PLR nor NLR alone was adequate to confirm or exclude the diagnosis of colorectal carcinoma or adenoma. The PLR value was 9.12 in the diagnosis analysis of

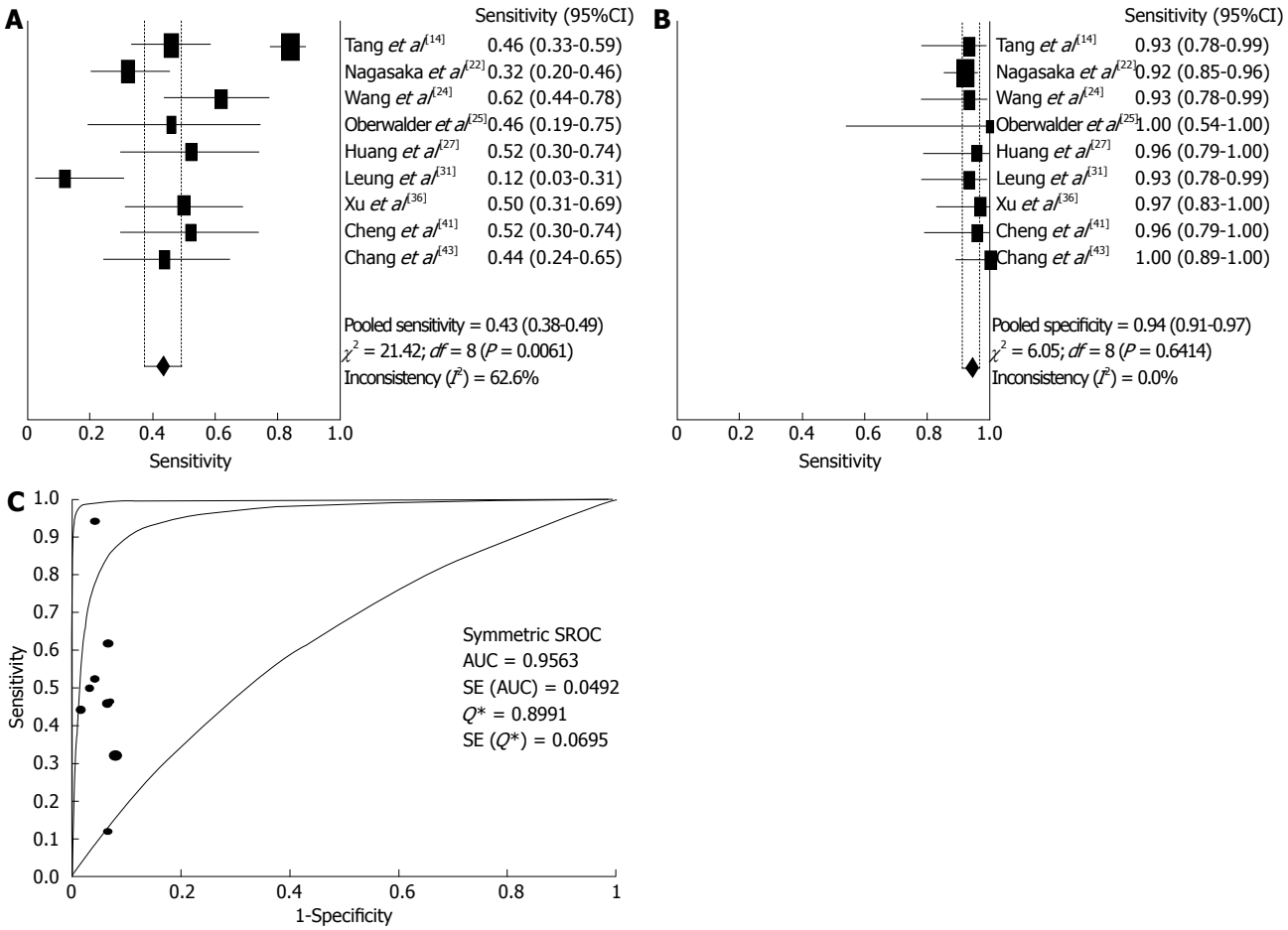

Figure 3 Forest plot of SFRP2 methylation in the diagnosis of colorectal adenomas. A: Plot and table of the sensitivity of SFRP2 for diagnosis of colorectal adenomas; B: Plot and table of the specificity of SFRP2 for diagnosis of colorectal adenomas; C: The symmetric summary receiver-operating characteristic of SFRP2 for diagnosis of colorectal adenomas.

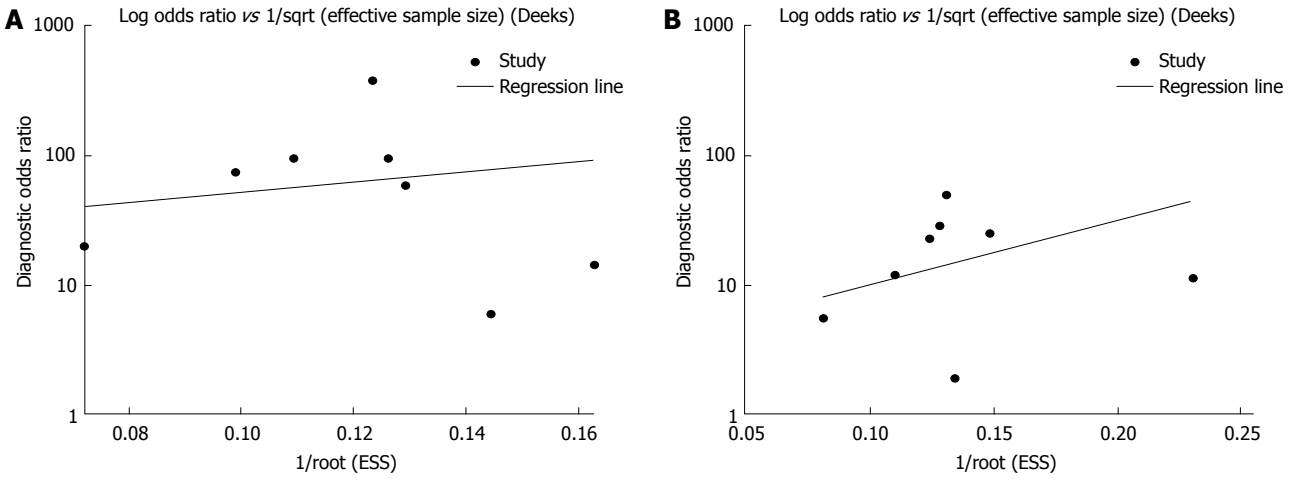

Figure 4 Assessment of the publication bias in the faecal SFPR2 methylation for the diagnosis of colorectal cancer (A) and adenomas (B). No significant publication biases were found in any of these studies (all $P > 0.05$).

CRC, which suggested that patients with a positive faecal SFRP2 methylation assay had a nine-fold chance of being diagnosed with CRC than non-CRC. Therefore, a colonoscopy was necessary for patients with a positive faecal SFRP2 methylation assay to confirm the diagnosis of CRC with high probability. On the other hand, an NLR of 0.24 in the diagnosis analysis of CRC suggested that if a faecal SFRP2 methylation assay result was negative, the probability rate of the individual having CRC was 24%. For the diagnosis of colorectal adenoma, a PLR of 5.99

Table 4 Weighted meta-regression on the diagnostic accuracy of the gene methylation assays

Covariate	Coefficient	SE	*P* value	RDOR	95%CI
QUADAS score[1]	-0.259	0.419	0.541	0.77	(0.33, 1.82)
Detection method[2]	0.101	0.377	0.790	1.11	(0.51, 2.40)
Blinded design[3]	-0.179	0.381	0.642	0.84	(0.38, 1.82)
Methylated genes[4]	0.302	0.394	0.449	1.35	(0.60, 3.03)

[1]Based on QUADAS score, studies were divided into those with higher quality (QUADAS score ⩾ 10) and those with lower quality (QUADAS score < 10); [2]Detection method, which was divided into qualitative and quantitative assay methods; [3]Blinded design: the study was included with or without blinded design; [4]Methylated genes, which were divided into single gene and combination genes. QUADAS: Quality Assessment for Studies of Diagnostic Accuracy was used to assess the quality of primary studies of diagnostic accuracy; SE: Standard error; RDOR: Relative diagnostic odds ratio.

suggested a moderate necessity to consider colonoscopy for patients with a positive faecal SFRP2 methylation assay to confirm the diagnosis of colorectal adenoma. Moreover, the NLR was 0.60 in the diagnosis analysis of colorectal adenoma. These data suggest that a negative faecal SFRP2 methylation assay result should not be used alone as a justification for denying or discontinuing the screening of colorectal adenomas.

An aberrant Wnt signalling pathway is an early event in 90% of colorectal carcinomas. SFRPs are secreted glycoproteins that antagonise Wnt signaling *via* different direct or indirect mechanisms. Thus, the role of SFRPs as a negative regulator of Wnt signaling may have important significance in tumourigenesis. These epigenetic events are involved in early steps of colon carcinogenesis, and changes in the status of DNA methylation are associated with early steps of the histologic progression of colon carcinoma. Our previous studies of CRC tissue showed that SFRP1 and SFRP2 were methylated in more than 80.6% of colorectal carcinomas[46]. Therefore, faecal SFRP2 methylation could be expected to be a biomarker for the screening of colorectal tumours. Although it cannot be generally used as a screening tool for the financial limited, the analysis of methylation markers offers a variety of new opportunities for developing biomarkers for colorectal tumours at the molecular level.

Our meta-analysis had several limitations. First, none of the included studies were multicentre or large-blinded, randomized, controlled trials. Second, conference abstracts and non-English and non-Chinese language studies were excluded, which might have led to publication bias. Third, studies on DNA methylation with statistical significance tend to be published and cited. Finally, due to the absence of case-mix difference analysis, smaller trials may show larger treatment effects than larger studies (*e.g.*, patients with only localised *vs* metastatic disease).

To sum up, stool-based DNA methylation has been shown to be highly discriminatory in the detection of colorectal tumours. Our results demonstrate that SFRP2 methylation, as a non-invasive modality, shows promise for the accurate detection of CRC; however, a large number of studies are required to further confirm the role of faecal SFRP2 methylation for the early and accurate CRC diagnosis.

COMMENTS

Background

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer-related deaths in Western countries. The diagnosis of CRC at early stages has great importance for reducing CRC mortality. Although significant advances have been achieved in diagnostic technologies, the current available modalities for diagnosing CRC remain suboptimal.

Research frontiers

DNA methylation often occurs during the early stages of colon tumours and has played an important role in oncology, especially in the early diagnosis of colorectal tumours. However, no consensus with regard to the role of stool methylation markers in colon tumours exists.

Innovations and breakthroughs

Stool methylation markers as an available non-invasive modality have high accuracy and sensitivity for the diagnosis of premalignant lesions of CRC. A few systematic reviews about the efficacy of stool methylation markers in colorectal tumour diagnosis exist. This article comprehensively assesses the accuracy of methylated genes in stool samples for diagnosing colorectal tumours.

Applications

Analysis of DNA methylation in stool samples may be used as a non-invasive test for the diagnosis of CRC, and SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis.

Terminology

Diagnostic odds ratio (DOR) reflects the relationship between the result of the diagnostic test and the disease. The summary receiver operation characteristic (SROC) curve displays the trade-off between sensitivity and specificity and represents a global summary of test performance. We used the Q-value, the intersection point of the SROC curve with a diagonal line from the left upper corner to the right lower corner of the receiver operation characteristic (ROC) space, which corresponds to the highest value of sensitivity and specificity for the test. The positive likelihood ratio (PLR) represents the value by which the odds of the disease increase when a test is positive, whereas negative likelihood ratio (NLR) shows the value by which the odds of the disease decrease when a test is negative.

Peer review

This study reviewed 34 trials to evaluate the accuracy of stool methylation genes for diagnosing colorectal tumours. Based on these analyses, the authors conclude that stool SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis. The analysis was carefully performed, the results were clearly presented and summarized, and valuable advice for early clinical diagnosis of colorectal tumours was provided.

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249 [PMID: 19474385 DOI: 10.3322/caac.20006]

2 **Schetter AJ**, Harris CC. Alterations of microRNAs contribute to colon carcinogenesis. *Semin Oncol* 2011; **38**: 734-742 [PMID: 22082759 DOI: 10.1053/j.seminoncol.2011.08.009]

3 **Markowitz SD**, Dawson DM, Willis J, Willson JK. Focus on colon cancer. *Cancer Cell* 2002; **1**: 233-236 [PMID: 12086859 DOI: 10.1016/S1535-6108(02)00053-3]

4 **Bosch LJ**, Carvalho B, Fijneman RJ, Jimenez CR, Pinedo HM, van Engeland M, Meijer GA. Molecular tests for colorectal cancer screening. *Clin Colorectal Cancer* 2011; **10**: 8-23 [PMID: 21609931 DOI: 10.3816/CCC.2011.n.002]

5 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96 [PMID: 18287387 DOI: 10.3322/CA.2007.0010]

6 **Heresbach D**, Manfredi S, D'halluin PN, Bretagne JF, Branger B. Review in depth and meta-analysis of controlled trials

on colorectal cancer screening by faecal occult blood test. *Eur J Gastroenterol Hepatol* 2006; **18**: 427-433 [PMID: 16538116 DOI: 10.1097/00042737-200604000-00018]
7 **Tagore KS**, Levin TR, Lawson MJ. The evolution to stool DNA testing for colorectal cancer. *Aliment Pharmacol Ther* 2004; **19**: 1225-1233 [PMID: 15191503 DOI: 10.1111/j.1365-2036.2004.02005.x]
8 **Berger BM**, Ahlquist DA. Stool DNA screening for colorectal neoplasia: biological and technical basis for high detection rates. *Pathology* 2012; **44**: 80-88 [PMID: 22198259 DOI: 10.1097/PAT.0b013e3283502fdf]
9 **Whiting P**, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003; **3**: 25 [PMID: 14606960 DOI: 10.1186/1471-2288-3-25]
10 **Ahlquist DA**, Taylor WR, Mahoney DW, Zou H, Domanico M, Thibodeau SN, Boardman LA, Berger BM, Lidgard GP. The stool DNA test is more accurate than the plasma septin 9 test in detecting colorectal neoplasia. *Clin Gastroenterol Hepatol* 2012; **10**: 272-277.e1 [PMID: 22019796 DOI: 10.1016/j.cgh.2011.10.008]
11 **Bosch LJ**, Oort FA, Neerincx M, Khalid-de Bakker CA, Terhaar sive Droste JS, Melotte V, Jonkers DM, Masclee AA, Mongera S, Grooteclaes M, Louwagie J, van Criekinge W, Coupé VM, Mulder CJ, van Engeland M, Carvalho B, Meijer GA. DNA methylation of phosphatase and actin regulator 3 detects colorectal cancer in stool and complements FIT. *Cancer Prev Res* (Phila) 2012; **5**: 464-472 [PMID: 22135045 DOI: 10.1158/1940-6207.CAPR-11-0315]
12 **Ahlquist DA**, Zou H, Domanico M, Mahoney DW, Yab TC, Taylor WR, Butz ML, Thibodeau SN, Rabeneck L, Paszat LF, Kinzler KW, Vogelstein B, Bjerregaard NC, Laurberg S, Sørensen HT, Berger BM, Lidgard GP. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology* 2012; **142**: 248-256; quiz e25-e26 [PMID: 22062357 DOI: 10.1053/j.gastro.2011.10.031]
13 **Azuara D**, Rodriguez-Moranta F, de Oca J, Soriano-Izquierdo A, Mora J, Guardiola J, Biondo S, Blanco I, Peinado MA, Moreno V, Esteller M, Capellá G. Novel methylation panel for the early detection of colorectal tumors in stool DNA. *Clin Colorectal Cancer* 2010; **9**: 168-176 [PMID: 20643622 DOI: 10.3816/CCC.2010.n.023]
14 **Tang D**, Liu J, Wang DR, Yu HF, Li YK, Zhang JQ. Diagnostic and prognostic value of the methylation status of secreted frizzled-related protein 2 in colorectal cancer. *Clin Invest Med* 2011; **34**: E88-E95 [PMID: 21463549]
15 **Baek YH**, Chang E, Kim YJ, Kim BK, Sohn JH, Park DI. Stool methylation-specific polymerase chain reaction assay for the detection of colorectal neoplasia in Korean patients. *Dis Colon Rectum* 2009; **52**: 1452-1459; discussion 1452-1459 [PMID: 19617759 DOI: 10.1007/DCR.0b013e3181a79533]
16 **Li M**, Chen WD, Papadopoulos N, Goodman SN, Bjerregaard NC, Laurberg S, Levin B, Juhl H, Arber N, Moinova H, Durkee K, Schmidt K, He Y, Diehl F, Velculescu VE, Zhou S, Diaz LA, Kinzler KW, Markowitz SD, Vogelstein B. Sensitive digital quantification of DNA methylation in clinical samples. *Nat Biotechnol* 2009; **27**: 858-863 [PMID: 19684580 DOI: 10.1038/nbt.1559]
17 **Melotte V**, Lentjes MH, van den Bosch SM, Hellebrekers DM, de Hoon JP, Wouters KA, Daenen KL, Partouns-Hendriks IE, Stessels F, Louwagie J, Smits KM, Weijenberg MP, Sanduleanu S, Khalid-de Bakker CA, Oort FA, Meijer GA, Jonkers DM, Herman JG, de Bruïne AP, van Engeland M. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. *J Natl Cancer Inst* 2009; **101**: 916-927 [PMID: 19535783 DOI: 10.1093/jnci/djp131]
18 **Ausch C**, Kim YH, Tsuchiya KD, Dzieciatkowski S, Washington MK, Paraskeva C, Radich J, Grady WM. Comparative analysis of PCR-based biomarker assay methods for colorectal polyp detection from fecal DNA. *Clin Chem* 2009; **55**: 1559-1563 [PMID: 19541867 DOI: 10.1373/clinchem.2008.122937]
19 **Hellebrekers DM**, Lentjes MH, van den Bosch SM, Melotte V, Wouters KA, Daenen KL, Smits KM, Akiyama Y, Yuasa Y, Sanduleanu S, Khalid-de Bakker CA, Jonkers D, Weijenberg MP, Louwagie J, van Criekinge W, Carvalho B, Meijer GA, Baylin SB, Herman JG, de Bruïne AP, van Engeland M. GATA4 and GATA5 are potential tumor suppressors and biomarkers in colorectal cancer. *Clin Cancer Res* 2009; **15**: 3990-3997 [PMID: 19509152 DOI: 10.1158/1078-0432.CCR-09-0055]
20 **Mayor R**, Casadomé L, Azuara D, Moreno V, Clark SJ, Capellà G, Peinado MA. Long-range epigenetic silencing at 2q14.2 affects most human colorectal cancers and may have application as a non-invasive biomarker of disease. *Br J Cancer* 2009; **100**: 1534-1539 [PMID: 19384295 DOI: 10.1038/sj.bjc.6605045]
21 **Kim MS**, Louwagie J, Carvalho B, Terhaar Sive Droste JS, Park HL, Chae YK, Yamashita K, Liu J, Ostrow KL, Ling S, Guerrero-Preston R, Demokan S, Yalniz Z, Dalay N, Meijer GA, Van Criekinge W, Sidransky D. Promoter DNA methylation of oncostatin m receptor-beta as a novel diagnostic and therapeutic marker in colon cancer. *PLoS One* 2009; **4**: e6555 [PMID: 19662090 DOI: 10.1371/journal.pone.0006555]
22 **Nagasaka T**, Tanaka N, Cullings HM, Sun DS, Sasamoto H, Uchida T, Koi M, Nishida N, Naomoto Y, Boland CR, Matsubara N, Goel A. Analysis of fecal DNA methylation to detect gastrointestinal neoplasia. *J Natl Cancer Inst* 2009; **101**: 1244-1258 [PMID: 19700653 DOI: 10.1093/jnci/djp265]
23 **Glöckner SC**, Dhir M, Yi JM, McGarvey KE, Van Neste L, Louwagie J, Chan TA, Kleeberger W, de Bruïne AP, Smits KM, Khalid-de Bakker CA, Jonkers DM, Stockbrügger RW, Meijer GA, Oort FA, Iacobuzio-Donahue C, Bierau K, Herman JG, Baylin SB, Van Engeland M, Schuebel KE, Ahuja N. Methylation of TFPI2 in stool DNA: a potential novel biomarker for the detection of colorectal cancer. *Cancer Res* 2009; **69**: 4691-4699 [PMID: 19435926 DOI: 10.1158/0008-5472.CAN-08-0142]
24 **Wang DR**, Tang D. Hypermethylated SFRP2 gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. *World J Gastroenterol* 2008; **14**: 524-531 [PMID: 18203283 DOI: 10.3748/wjg.14.524]
25 **Oberwalder M**, Zitt M, Wöntner C, Fiegl H, Goebel G, Zitt M, Köhle O, Mühlmann G, Ofner D, Margreiter R, Müller HM. SFRP2 methylation in fecal DNA--a marker for colorectal polyps. *Int J Colorectal Dis* 2008; **23**: 15-19 [PMID: 17639423 DOI: 10.1007/s00384-007-0355-2]
26 **Itzkowitz S**, Brand R, Jandorf L, Durkee K, Millholland J, Rabeneck L, Schroy PC, Sontag S, Johnson D, Markowitz S, Paszat L, Berger BM. A simplified, noninvasive stool DNA test for colorectal cancer detection. *Am J Gastroenterol* 2008; **103**: 2862-2870 [PMID: 18759824 DOI: 10.1111/j.1572-0241.2008.02088.x]
27 **Huang ZH**, Li LH, Yang F, Wang JF. Detection of aberrant methylation in fecal DNA as a molecular screening tool for colorectal cancer and precancerous lesions. *World J Gastroenterol* 2007; **13**: 950-954 [PMID: 17352030]
28 **Itzkowitz SH**, Jandorf L, Brand R, Rabeneck L, Schroy PC, Sontag S, Johnson D, Skoletsky J, Durkee K, Markowitz S, Shuber A. Improved fecal DNA test for colorectal cancer screening. *Clin Gastroenterol Hepatol* 2007; **5**: 111-117 [PMID: 17161655 DOI: 10.1016/j.cgh.2006.10.006]
29 **Abbaszadegan MR**, Tavasoli A, Velayati A, Sima HR, Vosooghinia H, Farzadnia M, Asadzedeh H, Gholamin M, Dadkhah E, Aarabi A. Stool-based DNA testing, a new noninvasive method for colorectal cancer screening, the first report from Iran. *World J Gastroenterol* 2007; **13**: 1528-1533 [PMID: 17461444]

30 **Zhang W**, Bauer M, Croner RS, Pelz JO, Lodygin D, Hermeking H, Stürzl M, Hohenberger W, Matzel KE. DNA stool test for colorectal cancer: hypermethylation of the secreted frizzled-related protein-1 gene. *Dis Colon Rectum* 2007; **50**: 1618-1626; discussion 1618-1626 [PMID: 17762966 DOI: 10.1007/s10350-007-0286-6]

31 **Leung WK**, To KF, Man EP, Chan MW, Hui AJ, Ng SS, Lau JY, Sung JJ. Detection of hypermethylated DNA or cyclooxygenase-2 messenger RNA in fecal samples of patients with colorectal cancer or polyps. *Am J Gastroenterol* 2007; **102**: 1070-1076 [PMID: 17378912 DOI: 10.1111/j.1572-0241.2007.01108.x]

32 **Petko Z**, Ghiassi M, Shuber A, Gorham J, Smalley W, Washington MK, Schultenover S, Gautam S, Markowitz SD, Grady WM. Aberrantly methylated CDKN2A, MGMT, and MLH1 in colon polyps and in fecal DNA from patients with colorectal polyps. *Clin Cancer Res* 2005; **11**: 1203-1209 [PMID: 15709190]

33 **Lenhard K**, Bommer GT, Asutay S, Schauer R, Brabletz T, Göke B, Lamerz R, Kolligs FT. Analysis of promoter methylation in stool: a novel method for the detection of colorectal cancer. *Clin Gastroenterol Hepatol* 2005; **3**: 142-149 [PMID: 15704048 DOI: 10.1016/S1542-3565(04)00624-X]

34 **Chen WD**, Han ZJ, Skoletsky J, Olson J, Sah J, Myeroff L, Platzer P, Lu S, Dawson D, Willis J, Pretlow TP, Lutterbaugh J, Kasturi L, Willson JK, Rao JS, Shuber A, Markowitz SD. Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. *J Natl Cancer Inst* 2005; **97**: 1124-1132 [PMID: 16077070 DOI: 10.1093/jnci/dji204]

35 **Müller HM**, Oberwalder M, Fiegl H, Morandell M, Goebel G, Zitt M, Mühlthaler M, Ofner D, Margreiter R, Widschwendter M. Methylation changes in faecal DNA: a marker for colorectal cancer screening? *Lancet* 2004; **363**: 1283-1285 [PMID: 15094274 DOI: 10.1016/S0140-6736(04)16002-9]

36 **Xu MH,** Cai KY, Tu Y. Stool DNA methylation analysis in the early diagnosis of colorectal cancer. *Chin J Clin Gastroenterol* 2012; **24**: 7-19 [DOI: 10.3870/lcxh.j.issn.1005-541X.2012.01.06]

37 **Kang YP,** Cao FA, Chang WJ, Lou Z, Wang Y, Wu LL, Fu CG, Cao GW. Stool DNA methylation in the screening early colorectal cancer. *J Gastrointest Surg* 2011; **14**: 52-561 [DOI: 10.3760/cma.j.issn.1671-0274.2011.01.017]

38 **Zhang JP,** Wang J, Gui YL, Zhu QQ, Xu ZW, Li JS. Stool DNA methylation in the diagnosis of colorectal cancer. *Chin Med J* 2011; **95**: 2482-2484 [DOI: 10.3760/cma.j.issn.0376-2491.2011.35.010]

39 **Fu L,** Sheng JQ, Meng XM, Meng MM, Jin P, Li AQ, Wu ZT, Li SR. Stool Vimentin methylation in the diagnosis of colorectal cancer. *J Gastroenterol Liver Dis* 2010; **19**: 601-603 [DOI: 10.3969/j.issn.1006-5709.2010.07.007]

40 **Ling ZA**, Chen LS, He CG. Stool P16 methylation in the diagnosis of colorectal cancer. *Colorectal Anus Surg* 2009; **15**: 144-148 [DOI: 10.3969/j.issn.1674-0491.2009.03.002]

41 **Cheng ZH.** Stool SFRP2 methylation in the diagnosis of colorectal cancer. *Shandong Yiyao Zazhi* 2007; **47**: 10-12 [DOI: 10.3969/j.issn.1002-266X.2007.06.004]

42 **Zhao HX,** Li QW, Dong WW, Duan XH, Zhu JH, Wang RL, Hao YX, Ye M, Xiao WH. Stool NDRG4 methylation in the diagnosis of colorectal cancer. *Zhongguo Yiyao Daobao* 2012; **9**: 29-31 [DOI: 10.3877/cma.j.issn.1674-0785.2012.10.012]

43 **Chang E**, Park DI, Kim YJ, Kim BK, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI, Kim HD, Kim DH, Kim YH. Detection of colorectal neoplasm using promoter methylation of ITGA4, SFRP2, and p16 in stool samples: a preliminary report in Korean patients. *Hepatogastroenterology* 2010; **57**: 720-727 [PMID: 21033217]

44 **Kim MS**, Lee J, Sidransky D. DNA methylation markers in colorectal cancer. *Cancer Metastasis Rev* 2010; **29**: 181-206 [PMID: 20135198 DOI: 10.1007/s10555-010-9207-6]

45 **Imperiale TF**, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004; **351**: 2704-2714 [PMID: 15616205 DOI: 10.1056/NEJMoa033403]

46 **Qi J**, Zhu YQ, Luo J, Tao WH. Hypermethylation and expression regulation of secreted frizzled-related protein genes in colorectal tumor. *World J Gastroenterol* 2006; **12**: 7113-7117 [PMID: 17131472]

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