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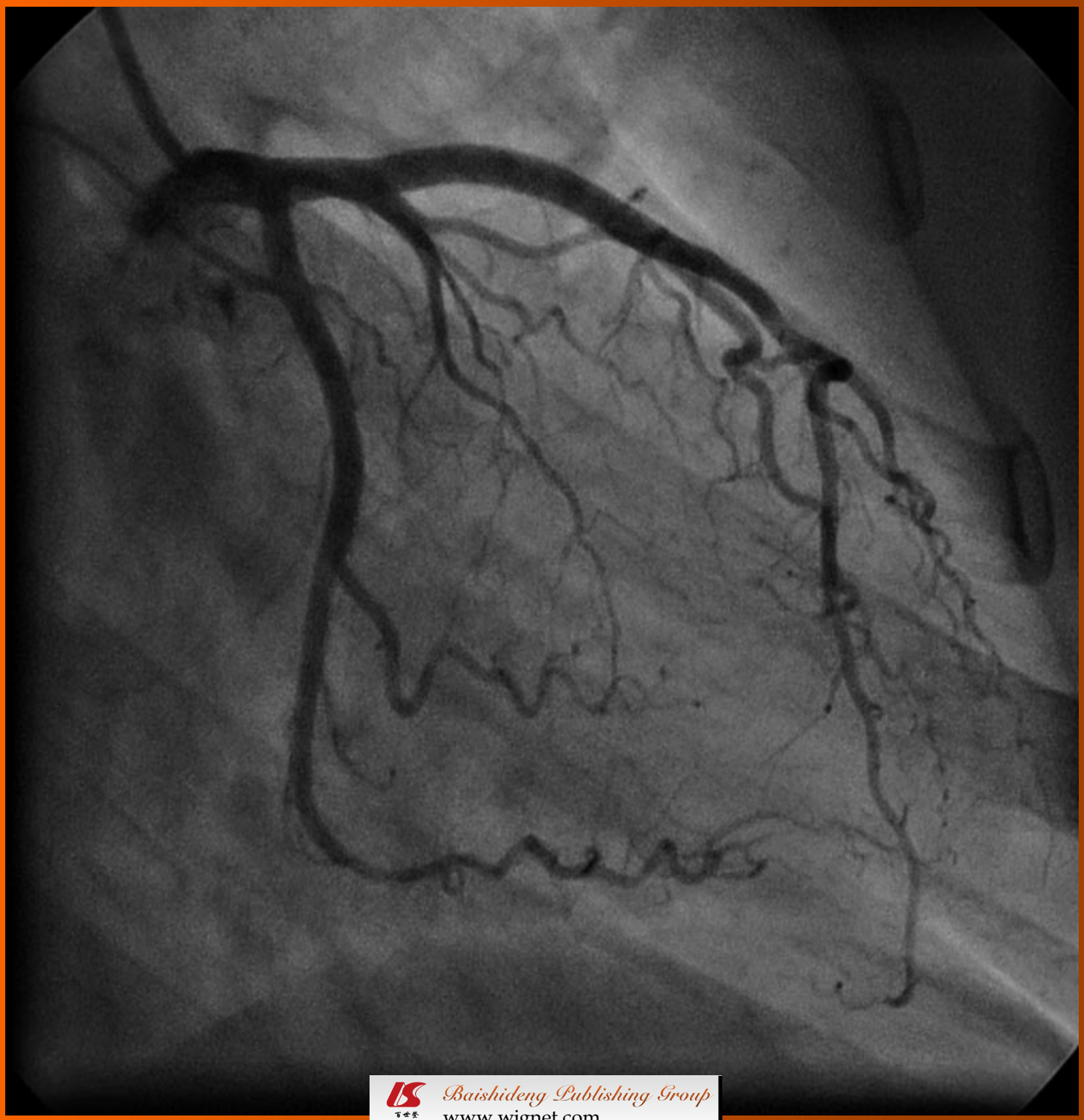

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EDITORIAL

JUPITER and satellites: Clinical implications of the JUPITER study and its secondary analyses

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Abstract

The justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER) study was a real breakthrough in primary cardiovascular disease prevention with statins, since it was conducted in apparently healthy individuals with normal levels of low-density lipoprotein cholesterol (LDL-C < 130 mg/dL) and increased inflammatory state, reflected by a high concentration of high-sensitivity C-reactive protein (hs-CRP ⩾ 2 mg/L). These individuals would not have qualified for statin treatment according to current treatment guidelines. In JUPITER, rosuvastatin was associated with significant reductions in cardiovascular outcomes as well as in overall mortality compared with placebo. In this paper the most important secondary analyses of the JUPITER trial are discussed, by focusing on their novel findings regarding the role of statins in primary prevention. Also, the characteristics of otherwise healthy normocholesterolemic subjects who are anticipated to benefit more from statin treatment in the clinical setting are discussed. Subjects at "intermediate" or "high" 10-year risk according to the Framingham score, those who exhibit low post-treatment levels of both LDL-C (< 70 mg/dL) and hs-CRP (< 1 mg/L), who are 70 years of age or older, as well as those with moderate chronic kidney disease (estimated glomerular filtration rate < 60 mL/min every 1.73 m^2) are anticipated to benefit more from statin treatment. Unlikely other statin primary prevention trials, JUPITER added to our knowledge that statins may be effective drugs in the primary prevention of cardiovascular disease in normocholesterolemic individuals at moderate-to-high risk. Also, statin treatment may reduce the risk of venous thromboembolism and preserve renal function. An increase in physician-reported diabetes represents a major safety concern associated with the use of the most potent statins.

Key words: Rosuvastatin; Primary prevention; Justification for the use of statins in prevention: An intervention trial evaluating rosuvastatin; Cardiovascular events; Mortality

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INTRODUCTION

Statins are still the cornerstone in the management of

dyslipidemia. Clinical trials demonstrated that statin therapy is associated with a significant reduction in cardiovascular morbidity and mortality when used for either primary or secondary prevention of cardiovascular events[1-3]. Interestingly, this benefit was so firmly confirmed in primary prevention studies involving patients with hypercholesterolemia, hypertension or diabetes mellitus, that the use of placebo in forthcoming statin trials has been considered as unethical[3].

The justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin (JUPITER) study made a step forward. This trial involved 17 802 apparently healthy individuals with normal low-density lipoprotein cholesterol (LDL-C) (< 130 mg/dL) and increased levels of high-sensitivity C-reactive protein (hs-CRP ⩾ 2 mg/L). The hypothesis whether rosuvastatin may decrease cardiovascular morbidity and mortality as compared with placebo in these subjects was tested[4]. JUPITER participants would not have qualified for statin therapy according to the existing guidelines for the management of dyslipidemia[5].

JUPITER revealed that rosuvastatin 20 mg/d decreased LDL-C levels by 50% and hs-CRP levels by 37%[4]. Also, rosuvastatin was associated with a significant decrease in the combined primary endpoint of myocardial infarction, stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular causes by 44% compared with placebo, after a median follow-up of 1.9 years[4]. Apart from this benefit in the primary endpoint, impressive reductions in the incidence of separate cardiovascular outcomes, including myocardial infarction (by 54%), stroke (by 48%) and revascularization for unstable angina (by 47%) compared with placebo were noted in the rosuvastatin-treated arm[4]. A reduction by 47% was also observed in the secondary combined endpoint of myocardial infarction, stroke, or death from cardiovascular causes[4].

Since the first release of JUPITER, many secondary analyses of this study have come to the light with regard to the results of the study into separate subgroups of the study population. Also, the effect of rosuvastatin treatment on outcomes not assessed in the initial part of the study was examined. In this paper, the most important *post hoc* analyses of the JUPITER study are reviewed by discussing major clinical implications derived from their results.

JUPITER IN SUBPOPULATIONS

Unlike other statin trials of primary prevention, JUPITER involved a quite different population, especially in terms of lipid profile and inflammation. Indeed, the population of JUPITER consisted of normocholesterolemic (LDL-C levels < 130 mg/dL), middle-aged (⩾ 50 years for men and ⩾ 60 years for women) subjects who exhibited increased hs-CRP levels (⩾ 2 mg/L)[4]. All individuals were statin-naïve and suitable for statin use in terms of safety parameters, while none had a history of serious medical conditions that could affect morbidity and mortality rates in the study period, including diabetes, uncontrolled hypertension or cancer[4].

From a total of 89 890 people screened, only 17 802 (19.8%) were eligible and finally enrolled in this study. Of the screening failures, more than half (i.e. 52.2%) were due to increased LDL-C levels > 130 mg/dL, while approximately one third (i.e. 36.1%) were due to low hs-CRP levels < 2 mg/L[4]. After 12 mo of treatment with rosuvastatin, LDL-C levels were decreased to the lowest level ever achieved in a primary prevention trial with the use of statins (i.e. 55 mg/dL).

JUPITER results according to cardiovascular risk classification

Several subanalyses explored the efficacy of rosuvastatin in reducing cardiovascular outcomes in different risk groups according to the Framingham or the Reynolds 10-year risk scores or the European systematic coronary risk (SCORE). In contrast to JUPITER participants who exhibited a 10-year risk of < 5%, those with a 10-year risk of ⩾ 5% experienced significant decreases in the relative risk for the primary endpoint associated with rosuvastatin treatment[6]. Rosuvastatin-associated benefits in all subgroups according to the Framingham or Reynolds 10-year risk classification (5%-10%, 11%-20% or > 20%) were comparable with the overall treatment effect observed in this study[6].

Among JUPITER participants, 6091 and 7340 subjects had a baseline estimated 10-year Framingham risk of 5%-10% and 11%-20%, respectively[6]. According to current guidelines these subjects are considered as a population of "intermediate" risk. JUPITER participants with a 10-year risk of 5%-10% or 11%-20% experienced significant absolute risk reductions[6]. Interestingly, absolute risk reductions increased with increasing level of global risk as assessed by either Framingham or the Reynolds risk scores[6]. For example, the estimated 5-year number needed to treat (NNT) of participants with a 5%-10% Framingham risk score was 40 (95% CI: 22-206), whereas in subjects with a Framingham risk score of 11%-20%, NNT was 18 (95% CI: 12-32)[6].

In another subanalysis, the results of JUPITER were evaluated in groups of participants who exhibited high global risk as defined by a 10-year Framingham risk score > 20% or SCORE ⩾ 5%[7]. Rosuvastatin treatment was associated with a relative risk reduction for the combined endpoint of myocardial infarction, stroke and cardiovascular death as compared with placebo in "high" risk subjects defined by either a Framingham score > 20% [hazard ratio (HR), 0.50; 95% CI: 0.27-0.93] or SCORE ⩾ 5% (HR, 0.57; 95% CI: 0.43-0.78)[7]. No differential change in the same endpoint was detected between subjects with a Framingham score above or below 20% as well as between individuals with a SCORE above or below 5%[7]. This benefit of rosuvastatin was also evident for the

primary endpoint among subjects with SCORE ≥ 5%[7]. In high-risk subjects, no heterogeneity for the combined endpoint of myocardial infarction, stroke and cardiovascular death was noted in subgroups by gender, age, race/ethnicity, the presence of hypertension or family history of cardiovascular disease, smoking status, baseline levels of high-density lipoprotein cholesterol (HDL-C) and hs-CRP[7]. Of interest, among high-risk patients, those who were obese at baseline, as defined by body mass index ≥ 30 kg/m^2, had less benefit from rosuvastatin treatment[7].

JUPITER results according to the lowering of lipids and hs-CRP

In another subanalysis, the effect of reductions in LDL-C and hs-CRP levels on trial event rates was assessed, by using pre-defined study cut-offs for both parameters[8]. No significant interaction between the overall efficacy of rosuvastatin and baseline concentrations of hs-CRP above or below 5 mg/dL and LDL-C above or below 100 mg/dL was noted in the JUPITER study[8]. Rosuvastatin-treated subjects who did not reach post-treatment levels of LDL-C < 70 mg/dL experienced no significant benefits as compared with placebo. In contrast, in individuals who attained LDL-C levels < 70 mg/dL a significant reduction in vascular event rates of 55% was noted[8]. A reduction in hs-CRP levels was also associated with clinical benefit. Thus, in patients with hs-CRP levels < 2 mg/L at the end of the study a significant decrease of 62% in cardiovascular events was observed[8]. This decrease was also significant, but modest (i.e. 31%) among individuals who did not achieve hs-CRP levels < 2 mg/L. All of the above variations were independent of the baseline levels of both LDL-C and hs-CRP[8]. Interestingly, subjects who achieved low concentrations of both LDL-C and hs-CRP (< 1 mg/L) after rosuvastatin treatment were at the lowest risk of cardiovascular events, shown by a decrease of 79% in risk[8].

There were similar findings in analogous assessments when other lipid parameters related to residual cardiovascular risk, including non-HDL-C levels above or below 100 mg/dL, apolipoprotein (apo)B target level above or below 80 mg/dL, or apoB to apoA1 ratio above or below 0.5, were put in the analysis as a substitute for LDL-C levels[8]. In all these analyses, participants achieving low concentrations of hs-CRP and low values of each lipid variable had a better clinical outcome compared with those who did not achieve the respective target[8].

To assess whether the rosuvastatin-associated clinical benefit for the primary endpoint was associated with HDL-C and apoA1 levels, study participants were divided into quartiles according to these parameters[9]. In the placebo group, LDL-C levels remained high and there was an inverse association of vascular risk with HDL-C and apoA1 levels. In contrast, this was not the case in the rosuvastatin-treated group in which LDL-C levels were decreased up to 55 mg/dL[9]. Therefore, HDL-C concentrations may not be predictive of residual cardiovascular risk among patients treated with potent statins who attain very low concentrations of LDL-C.

JUPITER results according to age

Compared with other statin trials, the JUPITER study involved a relatively older population (mean age, 66 years)[4]. In older populations there is a weaker association between total cholesterol levels and cardiovascular outcomes, possibly due to the existence of age-related comorbid conditions[10]. To date, there are limited data from randomized clinical trials regarding the efficacy of statins in the primary prevention of cardiovascular disease in the elderly[11]. A *post hoc* analysis of the JUPITER study focused on the efficacy of rosuvastatin to prevent cardiovascular events in study participants who were 70 years or older at recruitment[12]. Of 17 802 participants in the study, 5695 belonged to this age group. Despite being in a minority, older subjects accounted for 49% of the 393 confirmed primary endpoints in the trial[12]. Compared with younger participants, the older subjects exhibited a quite different risk profile, with female gender and hypertension being more prevalent among older persons than in younger ones. On the other hand, a lower percentage of subjects 70 years or older were obese or current smokers compared with younger subjects[12].

No differential effect between older and younger participants was detected with regard to post-treatment reductions of LDL-C and hs-CRP levels[12]. The analysis revealed that subjects 70 years or older may benefit more from rosuvastatin treatment, since the absolute risk reduction of the primary endpoint in this subpopulation was 48% greater than that observed in younger subjects[12]. Likewise, the NNT to prevent one primary endpoint was 24 for older individuals *vs* 36 for younger ones[12]. This difference was also evident for the composite endpoint of the primary endpoint, any death and venous thromboembolism (NNT 17 in older persons *vs* 27 in younger ones)[12]. No serious safety concerns from rosuvastatin use were raised in the older subpopulation compared with the younger one[12].

JUPITER results according to gender

Unlike secondary prevention trials, in primary prevention trials the reductions in coronary events associated with statin treatment were significant only in men, and not in women[13]. In the JUPITER study there was a predominance of the male gender over female (11 001 men *vs* 6801 women)[4]. Compared with male participants, female participants were older[14]. The different age-specific inclusion criterion (≥ 60 years in women and ≥ 50 years in men) could have accounted for this difference. Also, the prevalence of obesity, hypertension and metabolic syndrome was higher among women than men[14]. At baseline, women exhibited higher levels of hs-CRP than men (4.6 mg/dL *vs* 4.1 mg/dL), whereas no variation was observed in baseline LDL-C levels[14].

No gender-related variation with regard to post-treat-

ment changes in lipid parameters and hs-CRP levels was noted[14]. Also, the relative risk reduction for the primary endpoint with rosuvastatin was similar and statistically significant in both men and women[14]. Likewise, the reduction in overall mortality was quite similar between men and women (23% and 18%, respectively)[14]. Several differences were detected between men and women with regard to separate cardiovascular outcomes. For example, women experienced a greater risk reduction for revascularization/unstable angina than men (HR, 0.24; 95% CI: 0.11-0.51 for women *vs* HR, 0.63; 95% CI: 0.46-0.85 for men, $P = 0.01$ for heterogenicity)[14]. Nevertheless, unlike in men, no benefit for women was proved for several components of the primary endpoint, including myocardial infarction, stroke or death from cardiovascular causes. Of interest, in women a smaller reduction in nonfatal stroke was observed than in men ($P = 0.04$ for heterogeneity)[14].

Relative risk reductions in events were similar in women with either a Framingham risk score of 5%-10% or > 10% (HR, 0.44; 95% CI: 0.22-0.89 and HR, 0.57; 95% CI: 0.34-0.97, respectively)[14]. The results were also similar for men stratified by Framingham risk scores. However, event rates were low in women and men with a Framingham risk score < 5% as well as in those younger than 65 years[14]. Subgroup analysis revealed that women with a family history of premature coronary heart disease may benefit more from rosuvastatin treatment than those without. This variation was not evident for men[14].

From these findings, the JUPITER study was the first primary prevention study which demonstrated that men and women with elevated hs-CRP levels could experience similar benefits from statin treatment in the prevention of cardiovascular outcomes.

JUPITER results in patients with chronic kidney disease

Patients with chronic kidney disease (CKD) exhibit increased cardiovascular morbidity and mortality compared with individuals with more preserved renal function[15]. Evidence from randomized clinical trials failed to show any benefit of statin treatment in high-risk patients with severe renal failure undergoing maintenance hemodialysis[15]. In the primary prevention basis, the WOSCOPS (West of Scotland Coronary Prevention Study) reported no significant benefit from pravastatin treatment among subjects with moderate CKD[16]. Pravastatin-associated benefits were obvious only in individuals with estimated glomerular filtration rate (eGFR) ⩾ 60 mL/min every 1.73 m^2[16].

The JUPITER study included 3267 patients with CKD, as defined by eGFR < 60 mL/min every 1.73 m^2[17]. From those, the vast majority (3253 subjects) had stage 3 impairment (eGFR 30-59 mL/min every 1.73 m^2) while only 14 had stage 4 renal impairment (eGFR 15-29 mL/min every 1.73 m^2)[17]. Subjects with renal impairment were older, more likely to be female and have a family history of premature cardiovascular disease, and exhibited a worse lipid profile as well as increased hs-CRP levels compared with those with normal renal function[17]. Also, those subjects were at increased risk of developing the primary endpoint of the study (HR, 1.54; 95% CI: 1.23-1.92, $P = 0.0002$) as well as arterial revascularization (HR, 1.53; 95% CI: 1.13-2.08, $P = 0.008$) and the combined endpoint of myocardial, infarction, stroke and cardiovascular death (HR, 1.44; 95% CI: 1.08-1.92, $P = 0.02$) compared with subjects with preserved renal function[17]. The two groups did not differ with regard to all-cause mortality and rates of venous thromboembolism[17].

The reduction in the primary endpoint by rosuvastatin treatment compared with placebo was significant (HR, 0.55; 95% CI: 0.38-0.82, $P = 0.002$) in the group of individuals with moderate CKD and was comparable to that observed in subjects with preserved renal function[17]. Likewise, the efficacy of rosuvastatin to reduce the risk of all vascular events was similar between the two groups. All-cause mortality was the only exception, which was reduced more by rosuvastatin in moderate CKD subjects compared with subjects with normal renal function (44% *vs* 12%, P for interaction = 0.048)[17]. Of interest, patients with moderate CKD experienced a greater absolute risk reduction in the primary endpoint than subjects with preserved renal function (NNT 14 and 35, respectively)[17]. No differential effect of rosuvastatin was noted in the two groups with regard to decreases in LDL-C and hs-CRP levels. There was no difference between the two groups regarding safety[17].

JUPITER AND ALTERNATIVE OUTCOMES

Stroke

In primary prevention trials with the use of statins there were no significant decreases in the risk of stroke[18]. Therefore, this outcome was assessed separately in a secondary analysis of the JUPITER study[19]. Rosuvastatin was associated with a decrease in the risk of stroke by 48% compared with placebo, a rate which was similar also for nonfatal strokes[19]. A decrease by 51% associated with rosuvastatin treatment was noted for ischemic strokes, which accounted for the majority of all strokes[19]. Nevertheless, no effect of rosuvastatin treatment was noted with regard to the risk of hemorrhagic stroke or transient ischemic attacks[19].

Rosuvastatin-related benefits in the risk of stroke were similar in different groups according to age, sex, ethnicity, the presence of traditional risk factors for stroke, including age > 70 years, smoking, hypertension and a family history of premature stroke or a Framingham risk score > 10%[19]. As with other vascular outcomes, the greatest reduction in stroke risk was noted among those who achieved LDL-C levels < 70 mg/dL and hs-CRP < 2 mg/L[19].

Venous thromboembolism

There is still controversy with regard to the nature and

the shared pathways of venous and arterial thrombosis[20]. Also, it has not yet been defined whether treatment proven efficacious in the prevention of one condition may have consistent benefits for the other[20]. Statins exhibit many lipid-independent antithrombotic and anticoagulant effects[21]. To date, there are conflicting data from observational studies as to the effect of statin treatment on the risk of venous thrombosis[20]. In the JUPITER study, rosuvastatin treatment was associated with a significant decrease in the risk of pulmonary embolism or deep vein thrombosis by 43% compared with placebo[22]. Similar benefits of rosuvastatin were found when provoked (in patients with cancer, recent trauma, hospitalization or surgery) and unprovoked events of venous thromboembolism were examined separately[22]. Also, subjects at high risk for venous thromboembolism, including those aged > 70 years, body mass index > 30 kg/m^2 and increased waist circumference, exhibited a similar benefit associated with rosuvastatin treatment as in lower risk individuals[22]. No association between the risk of venous thromboembolism and baseline lipid levels was noticed[22].

Renal function

There is evidence that statins, through either their lipid-lowering properties or their pleiotropic effects, may preserve renal function and reduce proteinuria[15]. The effect of rosuvastatin on renal function was also assessed in the JUPITER study. After 12 mo of treatment with rosuvastatin, eGFR was marginally improved compared with placebo (66.8 mL/min every 1.73 m^2 *vs* 66.6 mL/min every 1.73 m^2, $P = 0.02$)[17]. This benefit was not evident in subjects with eGFR < 60 mL/min every 1.73 m^2, while it was more profound in individuals with eGFR ⩾ 60 mL/min every 1.73 m^2 at baseline[17].

Incidence of physician-reported diabetes

Increasing interest has been focussed on the effect that various statins may exert on glucose metabolism and the risk of diabetes[23]. In WOSCOPS pravastatin was associated with a decrease of 30% in the incidence of diabetes compared with placebo[24]. Nevertheless, JUPITER showed an increase in physician-reported diabetes in rosuvastatin-treated subjects compared with placebo-treated subjects (270 and 216 reports, respectively, $P = 0.01$)[4]. These events were not adjudicated by the endpoint committee of the trial. This result was documented despite no difference being observed between study groups for fasting glucose or newly diagnosed glycosuria[4]. However, a minimal increase in glycosylated hemoglobulin (Hb) was observed in the rosuvastatin group (5.9% *vs* 5.8%, $P < 0.001$)[4]. After this finding we have shown that rosuvastatin may be associated with a dose-dependent increase in insulin resistance among hyperlipidemic patients with impaired fasting glucose[25]. These findings were consistent with those of two recent meta-analyses of large-scale placebo-controlled and standard-care controlled trials, which, respectively, reported a 9% and 13% increased risk for incident diabetes associated with statin therapy[26].

Hb levels in patients with anemia

There is evidence suggesting that anemia of chronic disease may be associated with a functional iron deficiency mediated by immune mechanisms[27]. It has also been hypothesized that statins may contribute to an increase in Hb levels through immunomodulatory properties. In the JUPITER trial, Hb levels were determined at baseline and at the final visit in a secondary analysis which included study participants with anemia, as defined by Hb < 13 g/dL for men and < 12 g/dL for women[28]. A total of 369 women and 433 men met the inclusion criteria for this analysis. No difference between rosuvastatin and placebo was noted with regard to post-treatment changes in Hb levels[28]. Similar results were also found among patients with slightly worse anemia, as defined by Hb < 12.5 g/dL for men and < 11.5 g/dL for women[28].

CLINICAL IMPLICATIONS

Before JUPITER, studies showing a benefit of statins in primary prevention were limited to groups at high risk of cardiovascular disease, currently characterized as individuals with diabetes, hyperlipidemia, a family history of premature cardiovascular disease or those at high global cardiovascular risk. Since JUPITER, the potential efficacy of statins to prevent cardiovascular outcomes has been expanded to include normolipidemic individuals. The JUPITER population consisted of apparently healthy men and women with normal levels of LDL-C and an increased inflammatory state, indicated by high levels of hs-CRP. This disturbance was mainly attributed to obesity, smoking or metabolic syndrome, conditions which were frequent among the subjects. In this population there was no indication for statin treatment according to clinical practice guidelines. Interestingly, in JUPITER, rosuvastatin markedly decreased cardiovascular outcomes and moderately decreased mortality in this cohort.

Almost 20% of population screened in the JUPITER study fulfilled the criteria for inclusion in the study. When the eligibility criteria of JUPITER were analyzed in comparison with other community-based studies, such as the REGARDS (Reasons for Geographic and Racial Differences in Stroke) and the ARIC (Atherosclerosis Risk in Communities) studies, it was found that 21% and 18.2%, respectively, of each study could have been eligible for inclusion in the JUPITER study[29,30]. Another analysis suggested that approximately 6.5 million people in the United States could have been eligible for JUPITER[31]. Therefore, according to the JUPITER results a relatively high proportion of an age-matched population in the community could benefit from statin treatment in terms

of primary prevention. If these data are translated into practice, the current guidelines for statin use in primary prevention may dramatically change, leading to increased use of statin treatment. Furthermore, increasing interest will be applied in measuring hs-CRP in the screening of the normolipidemic population[32].

Secondary analyses of JUPITER highlighted those subjects who could benefit more from rosuvastatin treatment in terms of reduction in clinical outcomes. JUPITER suggested that no such benefit may be evident among individuals at low 10-year risk of < 5%. On the other hand, in subjects considered as of "intermediate risk", including those with a 10-year risk of 5%-10% or 11%-20%, a profound clinical benefit in the primary prevention of cardiovascular disease may be produced by statin treatment. This finding implies that this group of subjects, who were currently outside treatment guidelines according to their baseline LDL-C levels (i.e. < 104 mg/dL), might well be considered for statin therapy. Also, in such populations hs-CRP may comprise a useful tool for the reclassification of risk. In an analysis recently performed by Choudhry *et al*[33], it has been suggested that measuring hs-CRP levels may be valuable in order to identify patients in whom rosuvastatin treatment may be cost-effective in the primary prevention setting. Rosuvastatin treatment was proved cost-effective among JUPITER-eligible patients, especially in those with a Framingham risk score ⩾ 10%[33].

Finally, after the results of JUPITER were disseminated, the Canadian Cardiovascular Society changed its guidelines to include the measurement of hs-CRP, along with LDL-C and HDL-C levels, among otherwise healthy men and women at "intermediate risk"[34]. Of interest, the absolute risk reduction associated with statin treatment in intermediate risk subjects may be in parallel with their 10-year risk for cardiovascular disease. All patients at high risk as defined by a Framingham score of > 20% or SCORE ⩾ 5%, except obese subjects, may experience significant benefits from statin treatment in the reduction of cardiovascular outcomes.

Clinical benefit from statin use may also be associated with post-treatment decreases in LDL-C levels and hs-CRP levels among normocholesterolemic subjects with increased hs-CRP levels. The JUPITER study proposed LDL-C levels 70 mg/dL and hs-CRP 1 mg/L as the cut-off points below which major clinical benefit could be achieved. If alternative lipid parameters are to be assessed instead of LDL-C, the suggested cut-off points are < 100 mg/dL for non-HDL-C, < 80 mg/dL for apoB and < 0.5 for the ratio apoB to apoA1. In contrast, HDL-C levels may not predictive of residual vascular risk in patients treated with a potent statin who attain very low concentrations of LDL-C.

Despite similar reductions in LDL-C and hs-CRP levels, elderly normocholesterolemic individuals with increased inflammation may benefit more by statin treatment compared with younger subjects. Also, there may not be a gender-specific effect of statin treatment on the incidence of cardiovascular outcomes in normocholesterolemic subjects with increased hs-CRP concentrations. Statin-associated reductions in clinical outcomes in primary prevention of normocholesterolemic subjects with hs-CRP > 2 mg/L may be evident either in the clinical setting of moderate CKD. Of interest, mortality rates are more amenable to a reduction after statin treatment in those subjects compared with individuals with preserved renal function.

Furthermore, it has been hypothesized that the carriers of the KIF6 allele are preferentially affected by statin treatment compared with non-carrier subjects. This hypothesis was tested in a recent *post hoc* analysis of the JUPITER study. No significant association of KIF6 polymorphism and the efficacy of rosuvastatin treatment in primary prevention resulted from this study[35].

Stroke incidence may also be reduced by statin treatment in normocholesterolemic subjects with increased inflammation. Both lipid-lowering effects of statins and antiinflammatory properties of these drugs could contribute to this benefit. Furthermore, except for a reduction in the incidence of atherothrombotic events, antithrombotic effects of statins may also be associated with a decrease in the risk of venous thromboembolism. Also, statin treatment could contribute to an improvement in renal function of normocholesterolemic subjects with increased hs-CRP levels.

A potential increase in the incidence of diabetes should be a safety concern with statin treatment, especially when most potent drugs of the class are prescribed. However, this issue is currently under investigation. To this context, aggregation of clinical trials supports the notion that statins modestly increase the risk of diabetes. Because diabetes has been considered as a risk equivalent for vascular disease, these findings create a paradox whereby statin therapy may be withheld to avoid excess risk of diabetes, while representing the strongest cardiovascular risk reduction tool in diabetics[26]. A close monitoring of glucose homeostasis parameters in patients treated with statins is strongly recommended.

A future promising indication for statins, as effective antiinflammatory agents, is suggested by recent studies reporting a role of inflammation, and particularly of CRP, in enhanced atherogenicity among subjects with autoimmune inflammatory disorders, such as rheumatoid arthritis, systemic lupus erythematosus, familial Mediterranean fever and Behcet's disease[36].

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Calcium channels and iron uptake into the heart

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Abstract

Iron overload can lead to iron deposits in many tissues, particularly in the heart. It has also been shown to be associated with elevated oxidative stress in tissues. Elevated cardiac iron deposits can lead to iron overload cardiomyopathy, a condition which provokes mortality due to heart failure in iron-overloaded patients. Currently, the mechanism of iron uptake into cardiomyocytes is still not clearly understood. Growing evidence suggests L-type Ca^{2+} channels (LTCCs) as a possible pathway for ferrous iron (Fe^{2+}) uptake into cardiomyocytes under iron overload conditions. Nevertheless, controversy still exists since some findings on pharmacological interventions and those using different cell types do not support LTCC's role as a portal for iron uptake in cardiac cells. Recently, T-type Ca^{2+} channels (TTCC) have been shown to play an important role in the diseased heart. Although TTCC and iron uptake in cardiomyocytes has not been investigated greatly, a recent finding indicated that TTCC could be an important portal in thalassemic hearts. In this review, comprehensive findings collected from previous studies as well as a discussion of the controversy regarding iron uptake mechanisms into cardiomyocytes *via* calcium channels are presented with the hope that understanding the cellular iron uptake mechanism in cardiomyocytes will lead to improved treatment and prevention strategies, particularly in iron-overloaded patients.

Key words: Cardiomyocytes; L-type calcium channel; T-type calcium channels; Iron overload; Thalassemia

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INTRODUCTION

Iron (Fe) is an essential element for all living organisms and plays a central role in many Fe-containing proteins such as in iron storage proteins (ferritin and hemosiderin), energy metabolism (cytochromes, mitochondrial aconitase and Fe-S proteins of the electron transport chain), cellular respiration (hemoglobin and myoglobin), and DNA synthesis (ribonucleotide reductase)[1-3]. However, under iron overload conditions the regulatory mechanism which keeps the balance between iron uptake

and iron excretion could be disrupted, causing an elevation of non-transferrin bound iron (NTBI) in the plasma of iron-overloaded patients[4,5]. NTBI is toxic and participates in the production of harmful hydroxyl radicals, which could cause severe cellular damage and organ dysfunction[1,2,6,7].

An excess of plasma iron can lead to iron accumulation in many organs including the heart[5]. Excessive iron accumulation in the heart can cause cardiac cellular damage known as iron-overload cardiomyopathy. This cardiac complication causes 71% of all deaths in thalassemia major patients[8]. Although iron chelation therapy is widely used for treating iron overload patients, iron overload cardiomyopathy is still the most common cause of mortality in these patients[9,10]. Even though the fundamental mechanisms for excessive iron uptake in the heart have been investigated for decades, the precise mechanism underlying cardiomyocyte dysfunction induced by iron overload is not clearly understood. Although several NTBI transporters have been proposed and are responsible for cellular iron uptake, recent evidence suggests that calcium channels may play an important role as a portal for cardiac iron uptake[11].

In this review, the role of L-type Ca^{2+} channels (LTCC) as well as T-type Ca^{2+} channels (TTCC) as iron transporters into the heart are presented. The consistent findings as well as discrepancies of results among various studies on iron uptake into cardiomyocytes *via* these calcium channels under various conditions are comprehensively reviewed and discussed.

LTCCS AS A PORTAL FOR IRON UPTAKE INTO CARDIOMYOCYTES

The L-type Ca^{2+} channel is a voltage-gated ion channel that plays a central role in cardiac and smooth muscle contraction[12]. LTCCs are heterotetrameric polypeptide complexes that are composed of $\alpha1$, $\alpha2/\delta$, β, and, in some tissues, γ subunits[12]. The Ca^{2+} channel $\alpha1$ subunit (170-240 ku) is organized into four homologous motifs (I -IV), with six transmembrane segments (S1-S6)[12]. Recently, 10 $\alpha1$ subunit genes have been identified including $Ca_v1.1$ ($\alpha1S$), 1.2 ($\alpha1C$), 1.3 ($\alpha1D$), 1.4 ($\alpha1F$), $Ca_v2.1$ ($\alpha1A$), 2.2 ($\alpha1B$), 2.3 ($\alpha1E$), $Ca_v3.1$ ($\alpha1G$), 3.2 ($\alpha1H$), and 3.3 ($\alpha1I$). For LTCCs, these can be divided into 4 classes: $Ca_v1.1$ ($\alpha1S$), 1.2 ($\alpha1C$), 1.3 ($\alpha1D$), and 1.4 ($\alpha1F$). In cardiac muscles, only the $\alpha1C$ (dihydropyridine-sensitive) subunit is expressed in high levels and is also called a high-voltage-activated channel[12]. LTCCs can be found in the heart and are primarily used for Ca^{2+} transport as well as playing an important role in the electrical activity of the heart. However, previous studies have shown that LTCCs can also transport other divalent cations including Fe^{2+}[13-15].

Several findings have been shown to support the role of LTCC in myocardial iron transport[11,15]. A study in an iron loaded perfused rat heart showed that iron uptake was increased by the LTCC agonist, Bay K 8644 and iron uptake was inhibited by the LTCC blocker, nifedipine[15]. Oudit *et al*[16] demonstrated that treatments with LTCC blockers such as amlodipine and verapamil could lead to the inhibition of LTCC current in cardiomyocytes, reduced myocardial iron accumulation, decreased oxidative stress and improved survival in iron-loaded mice. In addition, iron overloaded transgenic mice with cardiac-specific overexpression of LTCC were shown to have increased myocardial iron accumulation and oxidative stress, resulting in impaired cardiac function in comparison with control mice[16]. Furthermore, since the LTCC does not contain iron responsive elements (IREs) in the LTCC mRNA, it is not regulated by cellular iron levels under an iron overload condition. As a result, L-type Ca^{2+} currents were not decreased in iron overload conditions[16], confirming that the expression of LTCC was not regulated by the IRE. Furthermore, it has been shown in iron overloaded rats that the LTCC blocker diazepam could reduce mortality from iron overload without inhibition of iron absorption or urinary iron excretion[17].

In addition to the heart, a previous study also demonstrated that LTCC blockers verapamil and amlodipine did not decrease iron accumulation in the liver of mice with iron overload, and hypothesized that this was due to the fact that hepatocytes express minimal levels of LTCC[16]. However, a recent study by Ludwiczek and colleagues demonstrated that the LTCC blocker nifedipine could reduce iron accumulation in the liver of wild-type mice, but had no effect in divalent metal transporter 1 (DMT1) deficient mice, suggesting that this effect of nifedipine-mediated modulation of iron transport is *via* DMT1[18]. Nevertheless, these findings suggest that nifedipine could possibly be beneficial in iron overload cardiomyopathy.

DISCREPANCIES IN FINDINGS ON IRON UPTAKE INTO CARDIOMYOCYTES *VIA* LTCC

It is important to realize that not all reports regarding the mechanisms of iron uptake *via* LTCC are consistent. Despite strong evidence supporting the role of LTCC as a route for NTBI transport in the heart, Parkes and colleagues demonstrated otherwise[19]. In cultured rat neonatal myocytes, they demonstrated that LTCC blockers (nifedipine, verapamil, and diltiazem) did not alter iron uptake in these cells[19]. Our recent findings also demonstrated that the LTCC blocker verapamil could not prevent iron uptake into cultured adult mouse cardiomyocytes[20].

Several reasons to explain these inconsistent results may be drawn from previous reports. Most studies that support the role of LTCC for iron uptake in cardiomyocytes used freshly prepared cardiomyocytes taken from isolated perfused hearts[15] or *in vivo*[16]. However, a report that failed to show the role of LTCC in iron uptake into cardiomyocytes used cultured cardiomyocytes[19,20].

In cultured cardiomyocytes, it is possible that LTCC

may not fully develop compared with isolated cardiomyocytes obtained from the heart. If fully developed, it is also possible that the LTCC in cultured cardiomyocytes may not function properly. Furthermore, the ages of cultured cardiomyocytes could have played a role in this discrepancy.

In the light of these inconsistent findings, it is possible that cardiomyocytes obtained from different methods may have different cellular characteristics and properties. All of these proposed hypotheses have not been tested and will need to be further investigated to elucidate the definite mechanism of iron uptake into the heart and resolve these existing discrepancies.

TTCC AS A PORTAL FOR IRON UPTAKE INTO CARDIOMYOCYTES

TTCC have three isoforms: $Ca_v3.1$ (α1G), 3.2 (α1H), and 3.3 (α1I) that are localized to the brain, kidney, and heart and are also called low-voltage-activated channels[21]. It has been shown that only $Ca_v3.1$ and $Ca_v3.2$ are expressed in the heart[21]. TTCCs have been reported to be functionally expressed only in embryonic hearts and disappear in adults[22]. TTCC can be found abundantly only in sinoatrial pacemaker cells and Purkinje fibers of many species in adult hearts and are important for the maintenance of pacemaker activity[21,23]. However, TTCC currents and expression have been demonstrated to reappear and play an important pathological role in diseased hearts with conditions such as ventricular hypertrophy[21,24,25] and post-myocardial infarction[26]. The increased TTCC expression has been shown to contribute to the progression of heart failure[21].

Growing evidence indicates that TTCC blockers could be beneficial in diseased hearts. Recently, Horiba and colleagues demonstrated that the blockade of Ca^{2+} entry into cardiomyocytes *via* TTCC using the TTCC blocker efonidipine could block signal transduction involved in cardiac hypertrophy[27]. In addition, a study in a mouse model of dilated cardiomyopathy has shown that a TTCC blocker could restore the resting membrane potential, and reduce the number of premature ventricular contractions and ventricular tachycardia, thus reducing the incidence of sudden death in these mice[28]. These findings suggest that TTCC blockade may be potentially useful for the prevention of sudden death in patients with heart failure[28]. It is known that iron overload conditions can lead to increased iron uptake into cardiomyocytes, resulting in cardiac hypertrophy and failure[29-32]. However, it is not known if TTCC blockers could be cardioprotective in this type of cardiomyopathy.

Recently, our study using cultured cardiomyocytes taken from the heart of thalassemic mice demonstrated that intracellular iron accumulation in cultured ventricular myocytes of thalassemic mice was significantly higher than in wild type (WT) cells[20]. These findings suggest that thalassemic cardiomyocytes could have pathways which can greatly uptake iron into the cells more than that in WT cells. In addition, under an iron overloaded condition, our results demonstrated that the TTCC blocker, efonidipine, could prevent iron uptake into cultured thalassemic cardiomyocytes[20]. Although efonidipine is not a selective TTCC blocker and could also block LTCC, its efficacy in blocking TTCC is greater than that of LTCC[21]. In that study, since verapamil could not prevent iron uptake when efonidipine could, these findings suggested that TTCC could play a significant role in iron uptake into cardiomyocytes in this thalassemic cardiomyocyte model[20]. Moreover, our microarray data demonstrated that the TTCC genes were up-regulated in thalassemic hearts, which is well correlated with the iron uptake results, suggesting that TTCCs could play an important role in iron uptake in thalassemic hearts, and that their re-expression could be due to the pathological state of a thalassemic heart itself or from the iron-overloaded condition, or both.

Since iron overload patients can develop cardiomyopathy and heart failure[33-35], it is important that the association between iron overload, TTCC expression/function and cardiac complications be determined. Future studies in both basic and clinical research are needed to warrant the clinical usefulness of TTCC blockers in the prevention and treatment of iron overload cardiomyopathy particularly in thalassemia patients.

CONCLUSION

Iron overload is a serious and fatal complication in many diseases including iron-overload cardiomyopathy in thalassemia patients. Although pathways for cellular iron uptake have been investigated for many decades, its mechanism is still not clearly understood. In the past few years, findings regarding new possible pathways for cellular iron uptake have been suspected, including LTCC and TTCC. However, their definite roles as iron transporters in cardiomyocytes are still debated. Understanding the mechanism by which iron enters cardiac cells is very important, since it will provide us with the knowledge to be used in developing better treatment and prevention strategies in iron overloaded patients.

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GUIDELINES CLINICAL PRACTICE

Non-invasive detection of vulnerable coronary plaque

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Abstract

Critical coronary stenosis have been shown to contribute to only a minority of acute coronary syndromes and sudden cardiac death. Autopsy studies have identified a subgroup of high-risk patients with disrupted vulnerable plaque and modest stenosis. Consequently, a clinical need exists to develop methods to identify these plaques prospectively before disruption and clinical expression of disease. Recent advances in invasive and non-invasive imaging techniques have shown the potential to identify these high-risk plaques. Non-invasive imaging with magnetic resonance imaging, computed tomography and positron emission tomography holds the potential to differentiate between low- and high-risk plaques. There have been significant technological advances in non-invasive imaging modalities, and the aim is to achieve a diagnostic sensitivity for these technologies similar to that of the invasive modalities. Molecular imaging with the use of novel targeted nanoparticles may help in detecting high-risk plaques that will ultimately cause acute myocardial infarction. Moreover, nanoparticle-based imaging may even provide non-invasive treatments for these plaques. However, at present none of these imaging modalities are able to detect vulnerable plaque nor have they been shown to definitively predict outcome. Further trials are needed to provide more information regarding the natural history of high-risk but non-flow-limiting plaque to establish patient specific targeted therapy and to refine plaque stabilizing strategies in the future.

Key words: Atherosclerotic plaque; Magnetic resonance imaging; Multidetector row computed tomography; Single photon emission computed tomography

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INTRODUCTION

Technological advances in cardiovascular imaging in parallel with significant development in biomedical science has changed the way we assess coronary atherosclerosis. Interestingly, two very separate but intermingled concepts have emerged. In the first concept, regardless of the extent of coronary atheroma and luminal stenosis (as observed by coronary angiography), coronary pressure measurement is used to evaluate functional ischemia of the myocardium supplied by the stenotic epicardial vessel. The decision to revascularize a stenotic epicardial vessel is based on the presence or absence of a flow-limiting trans-stenotic coronary pressure gradient. The usefulness of this concept has been clinically validated[1-3]. The sec-

ond concept evolved after reports that claimed that most acute coronary events and coronary thromboses form on angiographically non-obstructive atheroma[4-6]. Standard coronary angiography often fails to identify the culprit lesion of non trans-mural acute myocardial infarction (AMI)[7]. In addition, the plaque burden, its delineation and constituents cannot be assessed by coronary angiography. These potentially lethal but mechanically non-obstructive plaques were later labeled as high-risk plaques or vulnerable plaques[8].

The extent of underlying plaque burden causing plaque rupture is a contentious issue. Early, and in fact some recent studies, have shown that coronary thrombosis and AMI are directly proportional to the severity of the coronary stenosis[9]. However, in contrast to this notion, there is also substantial evidence in the literature to believe that coronary thrombosis can develop in as many as two-thirds of cases with non-obstructive, high-risk vulnerable coronary plaques[4-6]. Regardless of the extent of underlying coronary atheroma leading to acute coronary syndromes (ACS), it is well documented that high-risk vulnerable plaques exist and are prone to rupture[10]. These plaques are generally treated conservatively and the possibility of adaptive remodeling is routinely overlooked. Most currently available diagnostic tests are unable to predict the risk of thrombosis associated with any particular lesion in the coronary arteries. Consequently, a clinical need exists to develop new techniques that are capable of identifying vulnerable plaques before disruption occurs.

VULNERABLE PLAQUE

The composition of atherosclerotic plaque is heterogeneous by nature and contains: (1) fibrocellular components [extracellular matrix and smooth muscle cells (SMCs)]; (2) lipid-cellular components (crystalline cholesterol and cholesterol esters mixed with macrophages); (3) thrombotic components (platelets and fibrin); and (4) calcium[11-14]. Vulnerable plaques that result in rupture have been now well described as thin cap fibroatheroma (TCFA). The term vulnerable plaque was first instituted by Muller *et al*[10]. These plaques contain extensive necrotic lipid core and a thin fibrous cap (< 65 μm)[14,15]. The TCFA that is prone to rupture is deficient in SMCs but contains type 1 collagen and infiltrating active macrophages. These macrophages release matrix metalloproteinases (MMPs) 1, 8 and 13 that weaken the fibrous cap and consequently result in rupture[16]. About 65%-70% of all coronary thrombi result from plaque rupture. Although the term *thin* (< 65 μm) fibrous cap is generally accepted to define vulnerable plaques, some conflicting reports have used higher thresholds (> 200 μm) to describe vulnerable plaques[17,18]. It is important to realize that that the pathological description of vulnerable plaque lacks physiological data. The histological observations are made on static and inert tissue while plaque rupture is a more dynamic process as recently reported by Abela *et al*[19]. Cholesterol expands in volume when crystallizing from a liquid to a solid, potentially leading to rupture. In addition to plaque rupture, plaque erosion and calcified nodules can also result in thrombotic plaque disruption[20]. Erosive plaques usually have intimal thickening and are composed of fibrotic tissue with a thick fibrous cap. The thick fibrous cap in contrast to a thin cap contains an abundance of SMCs, proteoglycans and type Ⅲ collagen, but very few inflammatory cells[20]. Determination of plaque characteristics is important to appreciate the pathophysiological process of atherothrombosis, and may also provide us with a means to establish risk assessment for individual plaques in individual patients. Accordingly, imaging modalities are required to reliably evaluate plaque composition and thereby allow implementation of treatment strategies to prevent adverse coronary events.

INVASIVE IMAGING MODALITIES

Invasive techniques to determine the vulnerable plaques have received more attention. In particular three different aspects of vulnerable plaques have been investigated using different invasive technologies. The first technique focuses on imaging the microanatomy of the plaque to identify the plaque components, and includes high frequency intra-vascular ultrasound (IVUS), virtual histology-IVUS®, intravascular optical coherence tomography and intravascular magnetic resonance imaging (MRI). The second set of techniques is directed at measuring metabolic activity of the plaque to predict the risk of plaque disruption, and includes intravascular thermography and elastography. The third technique relies on measuring plaque chemical composition and detailed characterization by employing near infrared reflectance or Raman spectroscopy. Although it is likely that initial prospective identification of vulnerable plaque will first be achieved by one of these competing intra-coronary technologies, several drawbacks exist in the widespread use of these technologies. Most invasive imaging modalities are novel and therefore require specific training and highly skilled staff, they are expensive to run and consequently are not feasible for routine clinical application. Readers are directed to specific reviews on invasive imaging to detect plaque[21]. This article will focus on current non-invasive modalities available to detect vulnerable plaque with especial focus on computed tomography (CT), MRI and positron emission tomography (PET) imaging.

NON-INVASIVE IMAGING TECHNIQUES

By their very nature, invasive imaging techniques are undesirable, with a lower level of patient acceptability than non-invasive alternatives, and thus face significant hurdles if they are to be accepted into routine clinical practice[22-26]. Non-invasive imaging modalities, namely CT, MRI and scintigraphic nuclear imaging techniques may provide an alternative to invasive imaging, and have shown consider-

Table 1 Characteristics of the "ideal" non-invasive imaging modality

Patient-related factors	Technical factors
Absence of ionizing radiation	Rapid image acquisition
Spacious (minimize claustrophobia)	High spatial resolution
Suitable for all (not precluded by aneurysm clips/pacemaker leads)	High contrast resolution
Absence of breath-holding	High temporal resolution
Administration of extrinsic contrast agents unnecessary	Electrocardiogram and respiratory gating
Wide range of clinical indications	Not limited by cardiac arrhythmia
	Provides both anatomic and metabolic information
	Reproducible
	Accurate

able promise in recent studies[27-32]. Continued technological advances have bridged the gap between the accuracies of these non-invasive "modern" techniques and their "traditional" invasive counterparts, with the result that the former have been accepted into routine practice for an ever-increasing spectrum of clinical conditions and indications. One of the ways to achieve increased sensitivity is through the use of nanoparticle-based molecular imaging[27,33,34]. Nanoparticle-enhanced MRI or single photon emission CT (SPECT) can identify a thrombus by detecting fibrin, confirm the presence of an inflammatory process by detecting leukocyte and macrophage infiltration, and recognize plaque angiogenesis by detecting specific integrins involved in the formation of new blood vessels[35-38]. In addition, molecules can also be used to label specific cells both *in vivo* and *ex vivo* for imaging, for example, stem cells for cardiac regeneration and lymphocytes for specific tumors. Furthermore, and in contrast to invasive imaging, molecular imaging may also be used to provide local treatments by targeting with specific therapeutic molecules. Finally, nanoparticle-enhanced MRI and PET scanners may play an important role in the development of new drugs by providing an *in vivo* assessment of the desired molecular effect of the investigational drug. This information could be very useful when deciding whether expensive phase Ⅲ trials for new pharmaceutical agents are justified.

The "ideal" non-invasive imaging modality

In order to understand the relative strengths and weaknesses of modalities in current practice, it is important to first determine the characteristics of the "ideal" non-invasive imaging modality (Table 1). Such a non-invasive modality would combine patient acceptability, acceptable clinical indications, safety, speed and high technical specification to produce unequivocal objective data upon which future management could be confidently based. It is apparent that modern techniques, while far superior to their predecessors, fall considerably short of these model standards.

MRI

Rapid innovation and development in MR technology has allowed for widespread acceptance of cardiovascular MRI as a valuable non-invasive *in vivo* imaging modality for assessment of myocardial contractility, viability and valvular function. This is due in no small part to the commercial availability of increasingly more robust coil and gradient technology in combination with novel pulse sequence design. This technique exploits differences in proton density, proton mobility, water content, chemical composition, molecular motion and diffusion to allow for exquisite soft tissue depiction, a reflection of the high contrast resolution of MRI. Most versatile at field strengths of 1.5 Tesla, this modality fulfils several of the desirable characteristics outlined in Table 1, including absence of ionizing radiation and requirement for breath-holding or extrinsic contrast agents as well as facilitating electrocardiogram (ECG) gating with high spatial and contrast resolution.

Despite the technological advances referred to above, coronary imaging has until recent times been beyond the capabilities of MRI. This reflects the diminutive size of the coronary vessels and thus poor contrast/signal to noise ratios, a short-lived "rest period" during diastole during which coronary motion is minimized, temporal restrictions imposed by the patient's ability to suspend respiration and the resultant limited spatial resolution attainable during that time period. Several of these issues have been addressed with considerable success by the introduction of T2 preparation navigator-gated and -corrected 3-dimensional segmented techniques which, although time-consuming, allow for volumetric imaging of the coronary arteries in their entirety with subsequent multiplanar reconstruction. However, coronary MR angiographic techniques remain technically demanding and suboptimal to merit widespread acceptance into the clinical arena.

HIGH RESOLUTION MRI FOR CORONARY PLAQUE IMAGING

MR angiographic evaluation of coronary arterial luminal patency has traditionally proven challenging for the reasons outlined above. One can appreciate therefore why attempts at coronary plaque imaging add an additional level of complexity, further stressing the boundaries of MRI capability. High resolution imaging using T1, T2 and proton density weighting allows for identification of

Table 2 Selected molecular imaging agents in cardiovascular disease

Biological process	Agent	Target	Imaging platform
Plaque rupture/thrombosis	Gadolinium carrying perfluorocarbon and peptides (EP-2104R)	Fibrin	MRI
	Phage display nanoparticles generated by CEST ^{99m}Tc-apcitide	Glycoprotein Ⅱb/Ⅲa receptor	SPECT
Inflammation/apoptosis	MNP + specific antibodies	E-selectin	MRI
	Monocrystalline iron oxide	ICAM/VCAM	PET
	Cross-linked iron oxide ^{18}FDG	Macrophages glucose transporter-1, hexokinase	SPECT
	^{99m}Tc-annexin	Phosphatidylserine/caspases	SPECT
	^{99m}Tc-interleukin-2	Lymphocytes	
Angiogenesis	MNP + specific antibody ^{99m}NC100692	VCAM-1/integrin $\alpha_v\beta_3$	MRI
Myocardial infarction	MNP ^{111}Indium oxide	Stem cell labeling	MRI/SPECT

MNP: Paramagnetic nanoparticle; CEST: Chemical exchange saturation transfer; MRI: Magnetic resonance imaging; SPECT: Single photon emission computed tomography; PET: Positron emission tomography; ICAM: Intercellular adhesion molecule; VCAM: Vascular cell adhesion molecule; ^{18}FDG: Fluorine-18 fluorodeoxyglucose.

calcium which is hypointense on all imaging sequences, lipid which is T1- and PD-hyperintense and T2-hypointense, and fibrous tissue which produces increased signal intensity on PD-weighted imaging, while it is isointense on T1 and isointense-to-hyperintense on T2-weighted imaging. These multi-contrast signal intensity "signatures" allow for characterization of various plaque components and plaque morphology on high-resolution imaging and therefore assessment of plaque vulnerability. In order to have a null signal from blood within the adjacent coronary lumen and thereby maximize contrast resolution, real-time respiratory navigated black blood fast spin echo sequences are generally utilized. Real-time slice position correction has also been employed. The accuracy of this technique has been histologically confirmed *ex vivo*[39]. This technique has also been employed to assess coronary wall thickness, with validation in both animal models and humans[40-43]. Fayad *et al*[41] reported positive remodeling and significant coronary wall thickening in patients with coronary artery disease in comparison with control patients. Such approaches may prove useful for non-invasive coronary plaque burden measurement in the absence of ionizing radiation exposure.

MOLECULAR MR FOR PLAQUE IMAGING

Evaluation of coronary plaque at the molecular level requires the addition of targeted contrast agents (Table 2). Paramagnetic gadolinium chelates are the most commonly used extracellular contrast agent for MRI. Although in nature this metal has a short half-life, this has been compensated by novel gadolinium constructs with albumin, high-density lipoprotein and liposomes[27,33,44-47]. In addition to a longer half-life, these new generation gadolinium chelates have improved affinity for adjacent protons, allowing superior imaging of the adjoining tissue. This affinity for protons is referred to as relaxivity time, R1 for longitudinal relaxivity (adjacent tissue appears bright) and R2 for transverse relaxivity (adjacent tissue appears dark).

Magnetic iron oxide is another class of paramagnetic nanoparticle (MNP) that has been used as a molecular agent for detection of plaque characteristics. Newer generations of MNP [termed monodisperse iron oxide (MION)] are pretreated with polymer coatings that offer several advantages including better *in vivo* stability and the ability to target multiple molecules by allowing stable conjugation of a variety of ligands to the nanoparticle. In addition, a cross-linked derivative of MION (CLIO) can be conjugated to near infrared fluorochromes to allow dual modality imaging with fluorescence microscopy and MRI[48,49]. These magnetofluorescent nanoparticle have been used to target specific molecules *in vivo*[49-52]. Furthermore, a longer half-life, a high relaxivity time and a small diameter allow this nanoparticle to be a useful molecular agent for plaque and myocardial imaging[53-56]. In addition to imaging static cellular markers, MNPs can be used to identify dynamic cellular targets (specific enzymes, e.g. proteases, oxidases) by using novel magnetic relaxation switches[57,58]. These magnetic relaxation switches produce a change in relaxivity time (R2) of the nanoparticle by undergoing reversible modification in structure in the presence of a specific enzyme, which is then detected by T2-weighted MRI[57,58]. These magnetic relaxation switches hold significant potential for identifying a large set of proteins relevant to clinical cardiology including troponin, brain natriuretic peptide and C-reactive protein.

After administration, the molecule-targeting nanoparticles can approach the plaque either through the lumen of the coronary artery or through the vasa vasorum in the outer vessel wall. These molecular agents can detect cell surface markers and therefore identify cells of interest. The cell surface markers are detected by attaching a targeted ligand to the nanoparticle, while cells are identified after cellular uptake and internalization of treated nanoparticles. To target and identify specific peptides or proteins, phage display screening libraries are normally used. Molecular MR approaches to image vulnerable plaque has focused on plaque thrombosis, plaque lipid content, plaque inflammation and plaque angiogenesis. To date, these molecular agents have successfully targeted several plaque components, including fibrin, cellular markers, e.g. vascular cellular adhesion molecule (VCAM),

and angiogenesis markers, e.g. integrin $\alpha_v\beta_3$.

Imaging targets of plaque rupture

Fibrin deposition on the plaque surface is the first step after endothelial disruption following plaque rupture. Therefore targeting fibrin on the plaque surface can potentially identify high-risk plaques that are prone to disruption. The first fibrin-targeting agent was perfluorocarbon nanoparticles, which contained a liquid perfluorocarbon core and an encapsulating phospholipid monolayer. These nanoparticles have the advantage of carrying > 90 000 gadolinium atoms, hence allowing superior T1-weighted contrast enhancement[59]. Fibrin can also be targeted using phage display methods, whereby fibrin-targeted gadolinium-labeled peptides are used. These peptides (e.g. EP-1873) allow MR detection of fibrin deposits on the surface of ruptured plaques with good histological correlation in an animal model of coronary thrombosis[60,61]. The new generations of fibrin specific peptides (e.g. EP-2104R) are more selective for fibrin and have demonstrated superior targeting of a thrombus *in vivo*[62]. The main limitation of fibrin-specific peptides is the small number of gadolinium atoms (only 4 atoms) that can be attached at one time, hence requiring accumulation in sufficient quantities at the site of imaging. Nonetheless, more recent chemical exchange saturation transfer (CEST) technology is now applied to nanoparticles allowing generation of stronger MR signals. CEST contrast can originate from endogenous amide or hydroxyl protons or from exchangeable sites on exogenous CEST agents. In this technology, exchangeable protons transfer magnetization to the strong signal of bulk water after irradiation. CEST contrast agents include a liposome-based nanoparticle LIPOCEST, and other paramagnetic nanoparticles PARACEST[63-66]. *In vitro* fibrin clots are targeted with the use of anti-fibrin antibody formulated with perfluorocarbon nanoparticle PARACEST contrast agents[66].

Imaging cell surface markers

One of the foremost goals of molecular imaging is to detect the early stages of vulnerable plaque formation. Inflammation plays a critical role in the initiation and pathogenesis of atherosclerosis[67]. Exposure to inflammatory cytokines leads to over-expression of cell surface adhesion molecules especially E-selectin, intercellular adhesion molecule-1 and VCAM-1. These adhesion molecules mediate adhesion and migration of leukocytes along the endothelial surface to the inflammatory site. Therefore significant attention has been paid to develop probes that can detect these activated molecules on the surface of endothelial cells.

The expression of E-selectin has been targeted *in vivo* by generation of pegylated paramagnetic liposomes formulated with anti E-selectin antibody. E-selectin could be successfully imaged in the collared carotid arteries of apolipoprotein E-deficient apoE $^{-/-}$ mice as compared with controls[37]. Several generations of MNPs have been used to target VCAM-1. More recently, a MNP phage display with linear peptide based probe was used to successfully image *in vivo* VCAM-1 expression in the aortic roots of apoE $^{-/-}$ mice[52]. Furthermore, statin treatment of these mice blunted the imaging signal by reducing accumulation of the probe in the aortic root, thereby demonstrating sufficient dynamic range to detect a treatment effect. The specificity of the MNP VCAM-1 probe has also been evaluated in *ex vivo* human carotid endarterectomy samples. Incubation of the samples with this probe resulted in co-localization of VCAM-1-expressing cells and the MNP probe on immunohistochemistry, and resulted in a reduction T2 signal[68].

Imaging cellular targets in atherosclerotic plaque

Gadofluorine is a more lipophilic chelate of gadolinium and forms micelles due to their hydrophobic fluorinated side chain[69]. This probe has been shown to accumulate in lipid rich atherosclerotic plaques in hypercholesterolemic animal models[35,70]. High density lipoprotein (HDL) plays a key role in removing excess cholesterol from the plaques, and therefore may be a suitable candidate for transfer of nanoparticles into the plaque. HDL-like nanoparticles containing gadolinium have been developed to image the plaque *in vivo*. It has been shown that these HDL-like nanoparticles accumulate in atherosclerotic plaques after their intravenous injection[44]. The rate of this contrast uptake appears to be related to the lipid and macrophage content of the plaques in apoE $^{-/-}$ mice[27]. In addition to gadolinium chelates, CLIO-MNPs have been used to image macrophages in both animal and human atherosclerotic plaques with good histological correlation[54,55,71]. Molecular targeting of macrophages could identify the presence of inflammation in the vulnerable plaques[33,44,72]. MNP-enhanced MRI has been used in a clinical trial to assess the effect of statin dose on the level of macrophage accumulation in patients with carotid atheromatosis[73].

Imaging for angiogenesis

Growing atherosclerotic plaques initiate angiogenesis to meet their increased metabolic needs. The microvessels can cause intra-plaque hemorrhage, thereby converting these plaques to high risk and prone to rupture. New vessel formation starts from the vasa vasorum in the outer wall of the arteries and is related to the key mediator of new blood vessel formation, integrin $\alpha_v\beta_3$[36]. This integrins may represent an important molecular target for diagnosing and treating angiogenesis-related diseases. In this context, an antagonist of integrin $\alpha_v\beta_3$ has been used to induce tumor regression by targeting inhibition of neovascularization both in animals and humans[74,75]. A paramagnetic liposome containing anti-$\alpha_v\beta_3$ antibodies has been used to image this integrin in an animal model[38]. Moreover, the use of an $\alpha_v\beta_3$ integrin antagonist (fumagillin) in an animal model of atherosclerosis resulted in an anti-angiogenic effect without affecting pre-existing normal blood vessels[34,74]. Other potential investigative targets for angiogenesis include vascular en-

dothelial growth factors and integrin $\alpha_1\beta_5$.

MRI has several distinct advantages over other techniques including the absence of ionizing radiation, use of significantly less nephrotoxic contrast agent, and facilitation of high spatial resolution imaging with superb soft tissue characterization. These traits can be further enhanced by the use of specific and targeted contrast agents. Molecular imaging can help detect vulnerable plaques and may provide a risk assessment of these plaques. Furthermore, novel therapeutic nanoparticles are being developed to target these high risk plaques to provide local treatment.

CARDIAC CT

In contrast to MRI, cardiac CT incurs significant ionizing radiation exposure (10-20 mSv for retrospective gated techniques and 1-10 mSv for prospective "single phase" techniques). This modality allows for rapid data acquisition at high spatial resolution, invokes less patient anxiety given its spacious, short gantry, and has fewer contraindications than MRI. However, patient cooperation with breath-holding instructions and administration of potentially nephrotoxic contrast agents are pre-requisites for diagnostic imaging.

Electron beam CT (EBCT), which featured non-mechanical movement of the electron source and fixed detectors has been replaced in recent times by multidetector row CT (MDCT) during which both the radiation source and detectors rotate during patient motion through the CT gantry. More recently, the introduction of dual-source CT methodologies have allowed for coronary imaging without the need for β blockade. Retrospective ECG gating allows coupling of MDCT data with the corresponding phase of cardiac contraction, providing multiphasic data with superior temporal resolution when compared with EBCT. This initially incurred penalties by means of increased radiation exposure although many "low dose" protocols have since been developed and accepted into routine clinical practice.

MDCT systems allow for non-invasive characterization of different plaque components[76]. Calcium can be detected with high sensitivity and has become the established means for detection and quantification of coronary artery calcification[77]. MDCT can also be used to detect non-calcified plaque in both *ex-vivo*[78] and *in vivo*[79,80] studies. The assessment of calcification within the arterial wall may provide an independent risk factor for coronary artery disease, but it fails to identify high risk vulnerable plaques. Early studies using contrast enhanced 4-slice MDCT of coronary plaques demonstrated good correlation in differentiating between soft, intermediate and calcified plaques, as compared with IVUS[81]. In another study, 4-slice MDCT demonstrated that non-calcified plaque contributed more to total plaque burden in patients with AMI in comparison to patients with stable angina[28]. More recent studies have attempted to quantify the total volume of non-calcified atherosclerotic plaque using 64-slice MDCT. One such study evaluated 50 patients some 17 mo apart and documented a mean annualized increase of 22% in plaque volume[31]. Inter-observer variability for the quantification of non-calcified plaque volumes was found to be substantial. In a separate study the same investigators reported strong correlation between CT plaque attenuation, positive remodeling and lipid content of the plaque at contrast enhanced 64-slice CT[82]. The progression of atherosclerotic lesions resulting in focal change in luminal patency is referred to as vessel wall remodeling[83]. Positive arterial remodeling is an adaptive compensatory mechanism aiming to maintain luminal patency. In contrast, negative remodeling results in luminal narrowing irrespective of the plaque volume[84-86]. It has been shown in studies that both positive remodeling and plaque lipid content determine plaque vulnerability[84,87,88]. This hypothesis was further confirmed in another clinical study where 16/64-slice CT was performed in 38 patients with ACS and 33 patients with stable angina. The investigators reported high positive predictive value for plaque vulnerability in the presence of positive remodeling, non-calcified plaque < 30 HU, and spotty calcification[89]. Low CT attenuation, positive remodeling and spotty calcification were further shown to be associated with high risk plaque in 147 patients by 64-slice CT[90]. Other investigators have reported a 97% sensitivity for 64-slice CT when compared with IVUS in detecting plaque in 26 patients who underwent both investigations, although MDCT performed less optimally in differentiating between soft and fibrous plaque composition[32]. It has been reported that CT tends to overestimate the volume of atherosclerosis compared with IVUS[91]. In a more recent study, Pflederer *et al*[92] compared morphological features of plaque in patients with ACS and stable patients using contrast-enhanced coronary dual-source CT. The culprit lesions in patients with ACS were reported to have spotty calcification, low CT attenuation, large plaque volume and higher remodeling indices, as compared to control stable lesions.

There is little doubt that future generations of MDCT scanners will allow for improved coronary arterial plaque detection and characterization. Continuing improvements in spatial and temporal resolution, combined with innovative techniques such as dual-energy CT and the non-invasive nature of CT may pave the way to making this modality highly attractive for the identification of vulnerable plaque in the wider population. High definition detectors, multi-source tube-detector configurations and flat panel detectors are likely to feature prominently in the near future.

SCINTIGRAPHIC IMAGING

Scintigraphic techniques including SPECT and PET hold the potential for superior functional and molecular atherosclerotic imaging for prediction of the risk of plaque rupture. These techniques allow study of changes at a cellular and molecular level, and have been used for clini-

cal and research purposes to study myocardial perfusion, innervation, angiogenesis, gene expression and stem cell labeling. While such metabolic information is surplus to that provided by MRI and MDCT, scintigraphic imaging techniques are limited by relatively poor spatial and temporal resolution. Both SPECT and PET involve significant ionizing radiation exposures, and as a result, these techniques also fall considerably short of the "ideal" described earlier.

Imaging cellular targets in atherosclerotic plaque

Fluorine-18 fluorodeoxyglucose (^{18}FDG) is a glucose analog that becomes concentrated in metabolically active cells. It has been suggested that its uptake within the atherosclerotic plaque is proportional to the degree of inflammation and macrophage density[93]. Preclinical studies have suggested that PET scanning can detect ^{18}FDG accumulation within the atherosclerotic plaque[29]. Rudd *et al*[30] demonstrated that human carotid plaque inflammation can be imaged with ^{18}FDG-PET and that symptomatic plaques accumulate more ^{18}FDG than asymptomatic lesions. In addition, histological examination of the excised symptomatic plaques in this study revealed heavy macrophage infiltration. This finding confirms that inflammation is present to a greater degree in symptomatic plaques. In a separate study, Tawakol *et al*[94] also showed noticeable correlation between ^{18}FDG-PET *in vivo* signals and macrophage content on histological examination after carotid endarterectomy.

Imaging proteases

Matrix degrading MMPs present in the fibrous cap of the vulnerable plaque can also provide a surrogate marker of plaque instability. Activated MMPs cause proteolysis of the extracellular matrix of the fibrous cap causing plaque remodeling and rendering it susceptible to rupture. Specific radiotracers based on inhibitors of MMPs (^{123}I-HO-I-CGS 27023A) have been generated and tested successfully in animal models[95,96]. Increased levels of macrophage or leukocyte apoptosis in the atherosclerotic plaque may contribute to plaque instability.

Imaging apoptosis

Annexin V is an endogenous protein that binds to phosphatidylserine, a negatively charged membrane phospholipid externalized to the cell surface during early cell apoptosis. ^{99m}Tc radiolabeled annexin V uptake in animal plaque has shown a good correlation with apoptosis[97]. In another similar animal model, treatment with statins resulted in a reduction in ^{99m}Tc annexin V accumulation within the plaque signifying plaque stabilization[98]. Intracellular activation of enzyme caspases is responsible for initiation of apoptosis and progress is ongoing to develop intracellular radiotracers to target these enzymes.

Imaging vasoconstricting peptides

Endothelins are 21 amino acid vasoconstricting peptides produced primarily by the endothelial cells and play a critical role in vascular homeostasis. Endothelin has three isoforms (ET-1, ET-2, ET-3), that bind to two endothelin receptors ET_A and ET_B, the latter being present on vascular endothelial cells. Elevated levels of ET-1 have been implicated in several vascular pathological processes including atherosclerosis, stent restenosis, endothelial dysfunction and angiogenesis. Positron emitting ^{18}F-labeled ET-1 has shown good receptor affinity *in vitro*[99]. ^{99m}Tc-labeled ETs have been used *in vivo* with high uptake in atherosclerotic plaques in animal models[100]. In addition, specific radiolabeled antagonists of ET receptors have been demonstrated in animal studies[101].

SPECT or PET imaging could be used to identify unstable plaque and therefore may allow target treatment of high-risk plaques regardless of their angiographic appearances. The shortcomings of PET scanning are the need for ionizing radiation, substantial background uptake by the active myocardium and poor spatial resolution; however, this has been overcome with new technological advances combining CT with a PET scanner in new hybrid CT/PET devices. The combined molecular and anatomical imaging with SPECT or PET combined with CT, MRI or echocardiography may increase anatomical localization of the radiotracer signal. At present, imaging of moving coronary arteries with SPECT and PET represents a challenge.

WHY IS PLAQUE DETECTION IMPORTANT?

Detection of vulnerable plaque may help avert subsequent acute coronary syndrome by facilitating timely preventive regional and local therapies to the coronary arteries. Identification and aggressive medical treatment of these high-risk plaques can stabilize these plaques and potentially reduce the incidence of AMI and sudden cardiac death. To date there has been no prospective clinical data available upon which to develop treatment criteria for these plaques. There are several shortcomings in current vulnerable plaque detection techniques. Most invasive and non-invasive methods of vulnerable plaque detection lack sensitivity and specificity. In addition, the natural history of vulnerable plaque is unclear and until the dynamic progression of such lesions is defined, it will be difficult to implement any coherent proven treatment strategy. Furthermore, at present there are no data to prove that interventional treatment strategies with percutaneous coronary intervention for these high risk but asymptomatic plaques are superior to conventional medical treatment. In our opinion it is therefore essential to refine the available non-invasive techniques to assess the natural progression of vulnerable plaque. An established gold-standard may then be used in a longitudinal study in patients with established coronary artery disease to truly characterize this complex and dynamic process.

CONCLUSION

Significant progress has been made in the last decade to advance our understanding of the biology of atheroscle-

rosis. This has resulted in identification of vulnerable plaques that are prone to acute thrombotic complications, which can account for the suddenness of clinical presentation in the majority of patients with coronary artery disease. A number of different novel imaging modalities have been investigated to define the specific characteristics of vulnerable plaque. However, most of these techniques are still undergoing constant refinement and cannot reliably identify vulnerable plaque in the clinical setting. It is important to realize that plaque composition is not equal to plaque vulnerability. Most of the methodologies described in this review are able to detect particular components of plaque, for example lipids and calcium. However, at present there is no definitive evidence that *in vivo* plaque composition is directly related to plaque vulnerability nor that the observed characteristics of a plaque are related to outcome. Further research is required to increase the sensitivity and specificity of these modalities to more accurately predict adverse events in the context of high-risk plaque. In our investigation of vulnerable plaque, it is essential that we do not forget to treat cardiovascular risk factors that initiate endothelial dysfunction, which remains the earliest pathological signal of atherosclerosis. In addition, peripheral blood can contain unique cells and cytokines that may also help in identifying the general population at risk for new sudden cardiovascular events. Many unanswered questions remain, but there is a real clinical imperative to understand the natural history of these non-obstructive plaques in order to better implement preventive strategies in the future.

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REVIEW

Implications of discoveries from genome-wide association studies in current cardiovascular practice

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Abstract

Genome-wide association studies (GWAS) have identified several genetic variants associated with coronary heart disease (CHD), and variations in plasma lipoproteins and blood pressure (BP). Loci corresponding to *CDKN2A/CDKN2B/ANRIL*, *MTHFD1L*, *CELSR2*, *PSRC1* and *SORT1* genes have been associated with CHD, and *TMEM57*, *DOCK7*, *CELSR2*, *APOB*, *ABCG5*, *HMGCR*, *TRIB1*, *FADS2/S3*, *LDLR*, *NCAN* and *TOMM40-APOE* with total cholesterol. Similarly, *CELSR2-PSRC1-SORT1*, *PCSK9*, *APOB*, *HMGCR*, *NCAN-CILP2-PBX4*, *LDLR*, *TOMM40-APOE*, and *APOC1-APOE* are associated with variations in low-density lipoprotein cholesterol levels. Altogether, forty, forty three and twenty loci have been associated with high-density lipoprotein cholesterol, triglycerides and BP phenotypes, respectively. Some of these identified loci are common for all the traits, some do not map to functional genes, and some are located in genes that encode for proteins not previously known to be involved in the biological pathway of the trait. GWAS have been successful at identifying new and unexpected genetic loci common to diseases and traits, thus rapidly providing key novel insights into disease biology. Since genotype information is fixed, with minimum biological variability, it is useful in early life risk prediction. However, these variants explain only a small proportion of the observed variance of these traits. Therefore, the utility of genetic determinants in assessing risk at later stages of life has limited immediate clinical impact. The future application of genetic screening will be in identifying risk groups early in life to direct targeted preventive measures.

Key words: Genome-wide association studies; Cardiovascular disease; Lipids; Blood pressure

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mor-

bidity and mortality globally[1,2]. There is a concerted effort to reduce this disease burden, particularly that of coronary heart disease (CHD) and cerebrovascular disease in developed countries[3-5]. These range from primary preventive strategies targeted at risk factors through acute management and secondary prevention strategies[6-8]. Kahn *et al*[9] estimated that aggressive application of nationally recommended prevention activities for CVD would potentially add approximately 224 million quality adjusted life-years to the US adult population over the next 30 years and improve the average lifespan by at least 1.3 years.

CHD is the result of a combination of genetic and environmental factors. More than 200 risk factors have been associated with CHD and, among these low-density lipoprotein cholesterol (LDL-c) and blood pressure (BP) have been shown through randomized controlled trials to be causally related to CHD. A key factor in reducing the global burden of CVD is early prediction of disease to target preventive interventions. More personalised approaches to CVD prevention are attracting increasing interest. Whilst biomarkers and quantitative traits have been extremely useful in targeting primary prevention, the recent advances in genomics offer a smart option for predicting future risk of disease very early in life using the invariant nature of a genotype throughout an individual's life-span. For example, Cohen *et al*[10] demonstrated that a genetic variant resulting in a modest 28% reduction in LDL-c from birth results in an 88% reduction in the risk of CHD. Over the last 5 years, genome-wide association studies (GWAS) have revolutionised the discovery of common genetic variants associated with a range of diseases and traits.

There are three key characteristics of a genetic variant that determine its impact on the phenotype studied - (1) the frequency of the variant; (2) the effect size of the variant on the phenotype; and (3) the number of genetic variants acting on the phenotype. The "common disease common variant" hypothesis (CD:CV) is the model invoked to explain how genes influence common traits such as lipids, coronary artery disease (CAD) and BP[11]. This model proposes, using an evolutionary paradigm, that common disease is due to allelic variants with a frequency greater than 5% in the general population and small individual effect size[12]. The CD:CV framework requires population-wide genotyping of very large numbers of common genetic variants (Single Nucleotide Polymorphisms/SNPs) to determine which variants show significant association with the phenotype studied. Technological advances now allow reliable and high-throughput genotyping of hundreds of thousands of SNPs on a genome-wide scale[13]. Such studies employ large scale association mapping using SNPs, making no assumptions about the genomic location or function of the causal variant, and test the hypothesis that allele frequency differs between individuals with differences in phenotype. In most GWAS, emphasis is given to the "*P* value" for the association of genotype with disease risk, to reduce the potential for false positive association that arises when the association of hundreds of thousands to millions of markers are tested across the whole genome. The current popular method for multiple-test correction is the frequentist approach of adjusting for a number of independent tests - based on this, a significance level of 5×10^{-8} is commonly used, in populations of European ancestry for an overall genome-wide significance threshold of 0.05, adjusted for an estimated 1 million independent SNPs in the genome by the Bonferroni method[14]. It should be noted that the Bonferroni method is a fairly conservative correction method that may increase false negative rate. Other corrections like the False Discovery Rate or permutation testing can be used to set a different threshold. In this context, it is pertinent to recognise that the *P*-value is an index of a true positive signal and does not in any way reflect the predictive potential of the associated variant. The current gold standard of validity is multiple replication in independent samples. We review the implications of positive GWAS findings in current cardiovascular practice.

GWAS AND CHD

We summarise the GWAS results of CHD from nine case-control studies and three cohort studies[15-26] (Figure 1 and Table 1). The effect sizes (OR) of susceptibility alleles were modest and ranged from 1.05-2.0. Common variants in chromosome 9p21 were implicated in nine independent case-control studies[16-23,25] and in two cohort studies[15,25]. The most replicated SNPs at chromosome 9p21 were rs0757278 and rs13333049. The loci corresponding to *MTHFD1L*, initially identified in the Wellcome Trust Case Control Consortium (WTCCC) study[17], were later replicated in the German Family MI study[18] with genome-wide statistical significance. However, it did not reach genome-wide statistical significance in the combined analysis of ten different data sets in the study by Kathiresan *et al*[21]. Genetic loci corresponding to *CELSR2*, *PSRC1* and *SORT1* on chromosome 1p13.3 are identified in three independent studies[18,20,21].

GWAS AND LIPIDS

Aulchenko *et al*[27] studied total cholesterol (TC)-associated genetic markers and identified 11 loci significantly associated with the trait (Figure 2 and Table 2): these corresponded to *TMEM57*, *DOCK7*, *CELSR2*, *APOB*, *ABCG5*, *HMGCR*, *TRIB1*, *FADS2/S3*, *LDLR*, *NCAN* and *TOMM40-APOE*. Many of these genes are also implicated in other lipid traits. After screening the genome for common variants associated with plasma lipids in > 100 000 individuals of European ancestry, Teslovich *et al*[28] identified 39 novel loci associated with TC and replicated several other loci found to be associated with lipid traits in the previous GWAS.

Table 1 Single nucleotide polymorphisms associated with coronary heart disease in genome-wide association studies

Chromosome	SNP	Position	Sample size	MAF (%)	OR (95% CI)	*P* value	Proximal gene	Ref.
1	rs646776	109620053	9746/9746	81.0	1.17 (1.11-1.24)		*CELSR2*	[18,20,21]
	rs599839	109623689	2801/4582	-	1.29 (1.18-1.40)	4.05×10^{-9}	*PSRC1*	
	rs599839	109623689	1926/2938	80.8	1.20 (1.10-1.31)	1.30×10^{-5}	*SORT1*	
1	rs11206510	55268627	25538[1]	81.0	1.15 (1.10-1.21)		*PCSK9*	[21]
1	rs17465637	220890152	9746/9746	72.0	1.13 (1.08-1.18)		*MIA3*	[18,21]
			2801/4582	-	1.20 (1.12-1.30)	1.27×10^{-6}		
2	rs6725887	203454130	9746/9746	14.0	1.17 (1.11-1.23)		*WDR12*	[21]
2	rs2943634	226776324	2801/4582	37/32	1.21 (1.03-1.30)	1.60×10^{-7}	*Intergenic*	[18]
3	rs9818870	139604812	19407/21366	17.3/15.4	1.15 (1.11-1.19)	7.44×10^{-13}	*MRAS*	[23]
6	rs12526453	13035530	25538[1]	65.0	1.12 (1.08-1.17)		*PHACTR1*	[21]
6	rs6922269	151294678	2801/4582	30.0/26.0	1.23 (1.15-1.33)	2.90×10^{-8}	*MTHFD1L*	[18]
	rs6922269	151294678	1926/2938	29.4/25.3	1.17 (1.04-1.32)	1.50×10^{-5}		
6[2]	rs2048327	160783522	4976/4383	4.1/2.1	1.82 (1.57-2.12)	4.20×10^{-15}	*SLC22A3*	[22]
	rs3127599	160827124					*LPAL2*	
	rs7767084	160882493					*LPA*	
	rs10755578	160889728						
9	rs10757278	22114477	1607/6728	51.7/45.3	1.28 (1.22-1.35)	3.60×10^{-14}	*CDKN2A*	[15,16,18,19,21,22,23,25]
	rs10757274	22086055	-	25.3/20.4	1.33 (1.23-1.47)		*CDKN2B*	
	rs1333049	22115503	875/1644	54.0/48.0	1.33 (1.18-1.51)	3.40×10^{-6}		
	rs1333049	22115503	1926/2938	55.4/47.4	1.47 (1.27-1.70)	1.16×10^{-13}	*MTAP*	
	rs1333049	22115503	12004/28949	-	1.24 (1.20-1.28)			
	-		9746/9746	56.0	1.28 (1.23-1.33)			
	rs4977574	22088574	-	-	-			
	-		19407/21366	-	-			
	-		33282	-	1.20 (1.08-1.34)[3]			
	rs1333049	22115503						
10	rs1746048	44095830	9746/9746	84.0	1.14 (1.08-1.21)		*CXCL12*	[18,21]
	rs501120	44073873	2801/4582	-/-	1.33 (1.20-1.48)	9.46×10^{-8}		
12	rs2259816	119919970	19407/21366	37.4/35.8	1.08 (1.05-1.11)		*HNF1A-C12 or f43*	[23]
16	rs4329913	55462933	18245	-	1.29 (1.02-1.63)[3]		*CETP*	[26]
	rs7202364	55342891			0.76 (0.59-0.99)[3]			
19	rs1122608	11024601	25538[1]	75.0	1.15 (1.10-1.20)		*LDLR*	[20,21]
	rs6511720	11063306	1926/2938	90.2	1.29 (1.10-1.52)	6.70×10^{-4}		
19	rs4420638	50114786	1926/2938	20.9	1.17 (1.08-1.28)	1.00×10^{-4}	*APOE/C1/C4*	[20,21]
	rs4420638	50114786	14365/30576					
21	rs9982601	34520998	25538[1]	13.0	1.28 (1.23-1.33)		*MRPS6*	[21]
							SLC5A3	
							KCNE2	

[1]WTCC and GerMIFS I and GerMIFS II were added to the total sample; [2]Haplotype CCTC; [3]Hazard ratio per allele after adjustment for age and multiple risk factors. CELSR2: Cadherin EGF LAG seven-pass G-type receptor 2; PSRC1: Proline/serine-rich coiled-coil 1; SORT1: Sortilin 1; PCSK9: Proprotein convertase subtilisin/kexin type 9; MIA3: Melanoma inhibitory activity family member 3; WDR12: WD repeat protein 12; MRAS: Ras-related protein M-Ras; PHACTR1: Phosphatase and actin regulator 1; MTHFD1L: Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like; SLC22A3: Solute carrier family 22 (extraneuronal monoamine transporter), member 3; LPAL2: Lipoprotein; Lp(a)-like 2 pseudogene; LPA: Lipoprotein Lp(a); CDKN2A: Cyclin-dependent kinase inhibitor 2A; CDKN2B: Cyclin-dependent kinase inhibitor 2B; MTAP: Methylthioadenosine phosphorylase; CXCL12: Chemokine (C-X-C motif) ligand 12; HNF1A-C12: Hepatocyte nuclear factor-1 homeobox A; CETP: Cholesteryl ester transfer protein plasma; LDLR: Low density lipoprotein receptor; APOE/C1/C4: Apolipoprotein; MRPS6: Mitochondrial ribosomal protein S6; SLC5A3: Solute carrier family 5 (sodium/myo-inositol cotransporter) member 3; KCNE2: Potassium voltage-gated channel subfamily E member 2; SNP: Single nucleotide polymorphisms; MAF: Minor allele frequency; OR: Odds ratio.

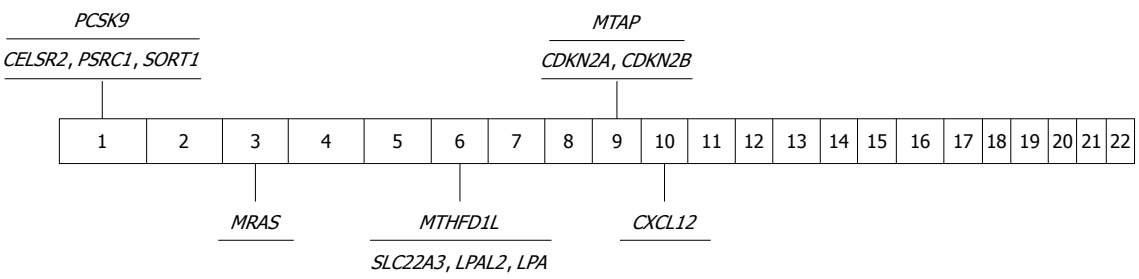

Figure 1 Significant genome-wide association study findings in coronary heart disease. CELSR2: Cadherin EGF LAG seven-pass G-type receptor 2; PSRC1: Proline/serine-rich coiled-coil 1; SORT1: Sortilin 1; PCSK9: Proprotein convertase subtilisin/kexin type 9; MRAS: Ras-related protein M-Ras; MTHFD1L: Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like; SLC22A3: Solute carrier family 22 (extraneuronal monoamine transporter), member 3; LPAL2: Lipoprotein, Lp(a)-like 2 pseudogene; LPA: Lipoprotein Lp(a); CDKN2A: Cyclin-dependent kinase inhibitor 2A; CDKN2B: Cyclin-dependent kinase inhibitor 2B; MTAP: Methylthioadenosine phosphorylase; CXCL12: Chemokine (C-X-C motif) ligand 12.

Table 2 Single nucleotide polymorphisms associated with total cholesterol identified through genome-wide association studies

Chromosome	Strongest SNP	Chromosome position	Sample size	MAF (average)	β	*P* value	Proximal gene	Ref.
1	rs10903129	25641524	22550	54	0.061	5.4×10^{-10}	*TMEM57*	[27]
1	rs1167998	62704220	17346	32	-0.073	6.4×10^{-10}	*DOCK7*	[27]
	rs108889353		19099	32	-0.079	3.7×10^{-12}		[27]
1	rs646776	109620053	17441	22	-0.128	8.5×10^{-22}	*CELSR2*	[27]
1	rs12027135	25648320	> 100000	45	-1.22	4.0×10^{-11}	*LDLRAP1*	[28]
1	rs7515577	92782026	> 100000	21	-1.18	3.0×10^{-8}	*EVI5*	[28]
1	rs2642442	219040186	> 100000	48	-1.36	5.0×10^{-14}	*IRF2BP2*	[28]
2	rs693	21085700	22500	52	-0.096	8.7×10^{-23}	*APOB*	[27]
2	rs6756629	43918594	17472	92	0.145	1.5×10^{-11}	*ABCG5*	[27]
2	rs7570971	135554376	> 100000	34	1.25	2.0×10^{-8}	*RAB3GAP1*	[28]
3	rs2290159	12603920	> 100000	22	-1.42	4.0×10^{-9}	*RAF1*	[28]
5	rs384662	35421429	20873	44	0.092	2.5×10^{-19}	*HMGCR*	[27]
	rs12916	74692295	> 100000	39	2.84	9.0×10^{-47}		[28]
5	rs6882076	156322875	> 100000	35	-1.98	7.0×10^{-28}	*TIMD4*	[28]
6	rs3177928	32520413	> 100000	16	2.31	4.0×10^{-19}	*HLA*	[28]
6	rs2814982	34654538	> 100000	11	-1.86	5.0×10^{-11}	*C6orf106*	[28]
6	rs9488822	116419586	> 100000	35	-1.18	2.0×10^{-10}	*FRK*	[28]
7	rs12670798	21573877	> 100000	23	1.43	9.0×10^{-10}	*DNAH11*	[28]
7	rs2072183	44545705	> 100000	25	2.01	3.0×10^{-11}	*NPC1L1*	[28]
8	rs6987702	126573908	17413	29	0.073	3.3×10^{-9}	*TRIB1*	[27]
8	rs2081687	59551119	> 100000	35	1.23	2.0×10^{-12}	*CYP7A1*	[28]
8	rs2737229	116717740	> 100000	30	-1.11	2.0×10^{-8}	*TRPS1*	[28]
10	rs2255141	113923876	> 100000	30	1.14	2.0×10^{-10}	*GPAM*	[28]
11	rs174570	61353788	20916	83	0.088	1.5×10^{-10}	*FADS2/3*	[27]
11	rs10128711	18589560	> 100000	28	-1.04	3.0×10^{-8}	*SPTY2D1*	[28]
11	rs7941030	122027585	> 100000	38	0.97	2.0×10^{-10}	*UBASH3B*	[28]
12	rs11065987	110556807	> 100000	42	-0.96	7.0×10^{-12}	*BRAP*	[28]
12	rs1169288	119901033	> 100000	33	1.42	1.0×10^{-14}	*HNF1A*	[28]
16	rs2000999	70665594	> 100000	20	2.34	3.0×10^{-24}	*HPR*	[28]
19	rs2228671	11071912	20910	88	0.158	9.3×10^{-24}	*LDLR*	[27]
19	rs2304130	19650528	20914	7	-0.153	2.0×10^{-15}	*NCAN*	[27]
19	rs2075650	50087459	17463	15	0.138	2.9×10^{-19}	*TOMM40-APOE*	[27]
	rs157580	50087106	20903	33	-0.09	5.1×10^{-17}		[27]
19	rs10401969	19268718	> 100000	7	-4.74	3.0×10^{-38}	*CILP2*	[28]
19	rs492602	53898229	> 100000	49	1.27	2.0×10^{-10}	*FLJ36070*	[28]
20	rs2277862		> 100000	15	-1.19	4.0×10^{-10}	*ERGIC3*	[28]
20	rs2902940	38524901	> 100000	29	-1.38	6.0×10^{-11}	*MAFB*	[28]

TMEM57: Transmembrane protein 57; DOCK7: Dedicator of cytokinesis 7; CELSR2: Cadherin, EGF LAG seven-pass G-type receptor 2; LDLRAP1: Low density lipoprotein receptor adaptor protein 1; EVI5: Ecotropic viral integration site 5; IRF2BP2: Interferon regulatory factor 2 binding protein 2; APOB: Apolipoprotein B; ABCG5: ATP-binding cassette sub-family G member 5; RAB3GAP1: RAB3 GTPase activating protein subunit 1 (catalytic); RAF1: v-raf-1 murine leukemia viral oncogene homolog 1; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; TIMD4: T-cell immunoglobulin and mucin domain containing 4; HLA: Human leukocyte antigen (HLA) complex; C6orf106: Chromosome 6 open reading frame 106; FRK: Fyn-related kinase; DNAH11: Dynein, axonemal, heavy chain 11; NPC1L1: NPC1 (Niemann-Pick disease, type C1, gene)-like 1; TRIB1: Tribbles Homolog-1 (*Trib1*); CYP7A1: Cytochrome P450, family 7, subfamily A, polypeptide 1; TRPS1: Trichorhinophalangeal syndrome 1; GPAM: Glycerol-3-phosphate acyltransferase; mitochondrial; FADS: Fatty acid desaturase; SPTY2D1: Suppressor of Ty, domain containing 1 (S. cerevisiae); UBASH3B: Ubiquitin associated and SH3 domain containing B; BRAP: BRCA1 associated protein; HNF1A: Hepatocyte nuclear factor-1 α; HPR: Haptoglobin-related protein; LDLR: Low density lipoprotein receptor; NCAN: Neurocan; TOMM40: Translocase of outer mitochondrial membrane 40 homolog; CILP2: Cartilage intermediate layer protein 2; ERGIC3: Endoplasmic reticulum-Golgi intermediate compartment protein 3; MAFB: V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; SNP: Single nucleotide polymorphisms; MAF: Minor allele frequency.

Prior to the publication of the meta-analysis of blood lipids conducted by Teslovich *et al*[28], 29 loci had been found to be associated with variation in high-density lipoprotein cholesterol (HDL-c) levels[20,27-39]. Teslovich *et al*[28] identified 31 novel loci associated with HDL-c with genome-wide significance. The most commonly-replicated loci are *LPL*, *LIPC*, *CETP*, *ABCA1*, *LIPG*, *APOA1/C3/A4/A5* and *GALNT2* (Figure 3 and Table 3). The *LIPC* locus has a set of common variants nearly 50 kb upstream of the gene, strongly associated with HDL-c and appearing to be independent of previously described variants that overlap the transcribed sequence of the gene. SNPs close to the mevalonate kinase-methylmalonic aciduria cblB type (*MMAB*) locus were found to be associated with HDL-c initially by Willer *et al*[20] and later confirmed by Kathiresan *et al*[29].

GWAS have identified several genetic loci associated with LDL-c (Figure 4 and Table 4)[20,27-32,34,36,40], such as the study by Teslovich *et al*[28] which identified 22 novel and 25 previously implicated loci. *CELSR2-PSRC1-SORT1* and *PCSK9* loci on chromosome 1, *APOB*, *HMGCR*, *NCAN-CILP2-PBX4*, *LDLR*, *TOMM40-APOE*, and *APOC1-APOE* were the most commonly-replicated loci in LDL-c. Several of these loci were also associated with

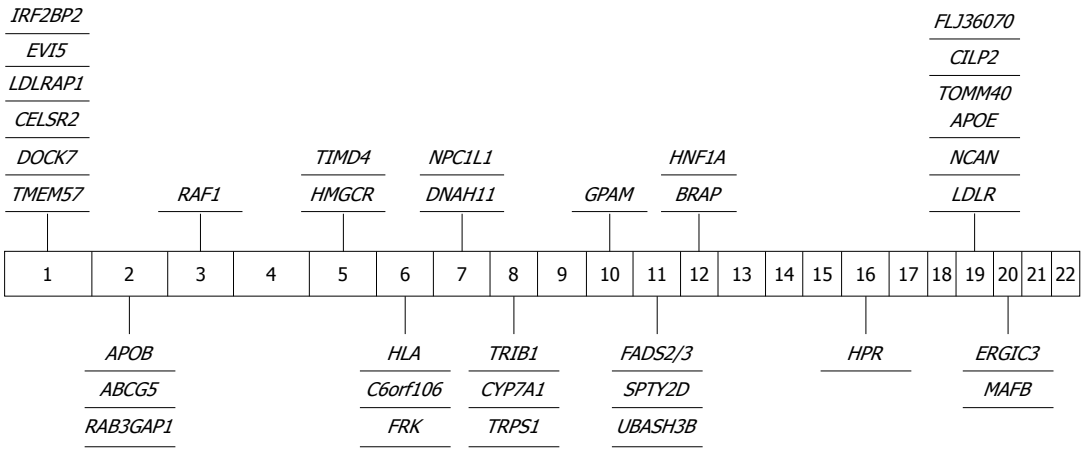

Figure 2 Significant genome-wide association study findings in total cholesterol. TMEM57: Transmembrane protein 57; DOCK7: Dedicator of cytokinesis 7; CELSR2: Cadherin, EGF LAG seven-pass G-type receptor 2; LDLRAP1: Low-density lipoprotein receptor adaptor protein 1; EVI5: Ecotropic viral integration site 5; IRF2BP2: Interferon regulatory factor 2 binding protein 2; APOB: Apolipoprotein B; ABCG5: ATP-binding cassette sub-family G member 5; RAB3GAP1: RAB3 GTPase activating protein subunit 1 (catalytic); RAF1: V-raf-1 murine leukemia viral oncogene homolog 1; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; TIMD4: T-cell immunoglobulin and mucin domain containing 4; HLA: Human leukocyte antigen (HLA) complex; C6orf106: Chromosome 6 open reading frame 106; FRK: Fyn-related kinase; DNAH11: Dynein, axonemal, heavy chain 11; NPC1L1: NPC1 (Niemann-Pick disease; type C1, gene)-like 1; TRIB1: Tribbles Homolog-1 (*Trib1*); CYP7A1: Cytochrome P450, family 7, subfamily A, polypeptide 1; TRPS1: Trichorhinophalangeal syndrome 1; GPAM: Glycerol-3-phosphate acyltransferase, mitochondrial; FADS: Fatty acid desaturase; SPTY2D1: Suppressor of Ty, domain containing 1 (S. cerevisiae); UBASH3B: Ubiquitin associated and SH3 domain containing B; BRAP: BRCA1 associated protein; HNF1A: Hepatocyte nuclear factor-1 α; HPR: Haptoglobin-related protein; LDLR: Low-density lipoprotein receptor; NCAN: Neurocan; TOMM40: Translocase of outer mitochondrial membrane 40 homolog; CILP2: Cartilage intermediate layer protein 2; ERGIC3: Endoplasmic reticulum-Golgi intermediate compartment protein 3; MAFB: V-maf musculoaponeurotic fibrosarcoma oncogene homolog B.

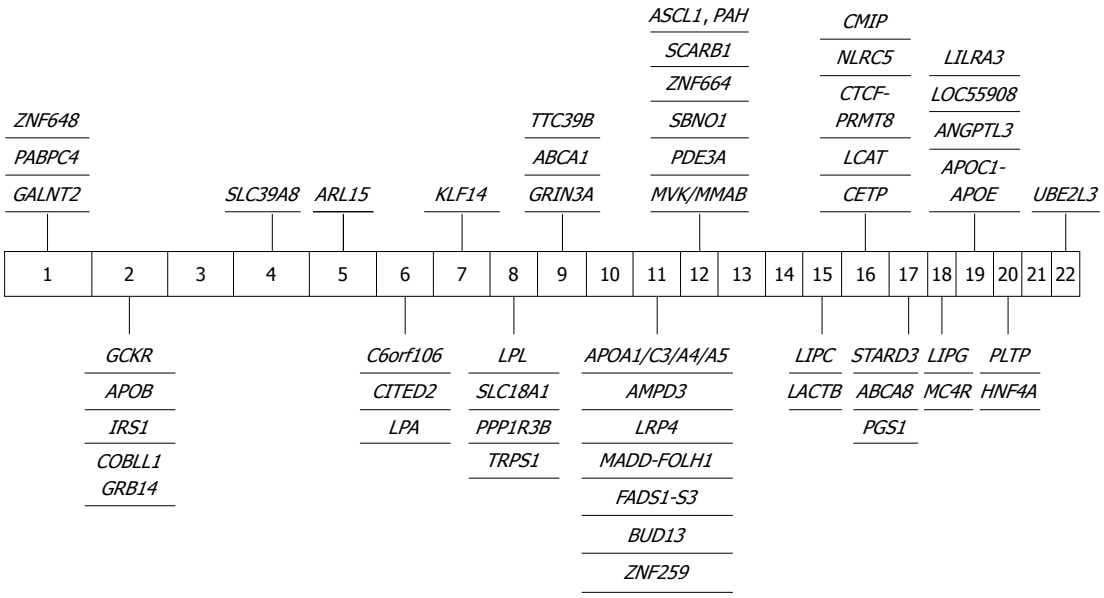

Figure 3 Significant genome-wide association study findings in high-density lipoprotein cholesterol. GALNT2: N-acetylgalactosaminyltransferase 2; PABPC4: Poly(A) binding protein; cytoplasmic 4 (inducible form); ZNF648: Zinc finger protein 648; GCKR: Glucokinase (hexokinase 4) regulator; APOB: Apolipoprotein B; IRS1: Insulin receptor substrate 1; COBLL1: COBL-like 1; GRB14: Growth factor receptor-bound protein 14; SLC39A8: Solute carrier family 39 (zinc transporter) member 8; ARL15: ADP-ribosylation factor-like 15; C6orf106: Chromosome 6 open reading frame 106; CITED2: Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain 2; LPA: Lipoprotein, Lp(a); KLF14: Kruppel-like factor 14; LPL: Lipoprotein lipase; SLC18A1: Solute carrier family 18 (vesicular monoamine) member 1; PPP1R3B: Protein phosphatase 1, regulatory (inhibitor) subunit 3B; TRPS1: Trichorhinophalangeal syndrome 1; GRIN3A: Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A; ABCA1: ATP-binding cassette; sub-family A (ABC1) member 1; TTC39B: Tetratricopeptide repeat domain 39B; ABCA1: ATP-binding cassette, sub-family A (ABC1) member 1; APOA1: Apolipoprotein A-I; AMPD3: Adenosine monophosphate deaminase 3; LRP4: Low-density lipoprotein receptor-related protein 4; MADD-FOLH1: MAP-kinase activating death domain- folate hydrolase (prostate-specific membrane antigen) 1; FADS1-S3: Fatty acid desaturase 1; BUD13: BUD13 homolog; ZNF259: Zinc finger protein 259; MVK: Mevalonate kinase; MMAB: Methylmalonic aciduria (cobalamin deficiency) cblB type; PDE3A: Phosphodiesterase 3A; SBNO1: Strawberry notch homolog 1; ZNF664: Zinc finger protein 664; SCARB1: Scavenger receptor class B member 1; ASCL1: Achaete-scute complex homolog 1; PAH: Phenylalanine hydroxylase; LIPC: Hepatic lipase; LACTB: Lactamase β; CETP: Cholesteryl ester transfer protein plasma; LCAT: Lecithin-cholesterol acyltransferase; CTCF: CCCTC-binding factor (zinc finger protein); PRMT8: Protein arginine methyltransferase 8; NLRC5: NLR family CARD domain containing 5; STARD3: StAR-related lipid transfer (START) domain containing 3; ABCA8: ATP-binding cassette; sub-family A (ABC1) member 8; PGS1: Phosphatidylglycerophosphate synthase 1; LIPG: Lipase endothelial; MC4R: Melanocortin 4 receptor; APOC1: Apolipoprotein C-I; APOE: Apolipoprotein E; ANGPTL3: Angiopoietin-like 3; LILRA3: Leukocyte immunoglobulin-like receptor, subfamily A (without TM domain) member 3; PLTP: Phospholipid transfer protein; HNF4A: Hepatocyte nuclear factor 4 α; PLTP: Phospholipid transfer protein; UBE2L3: Ubiquitin-conjugating enzyme E2L 3.

Table 3 Single nucleotide polymorphisms associated with high-density lipoprotein cholesterol identified through genome-wide association studies

Chromosome	Strongest SNP	Chromosome position	Sample size	MAF (average)	Change in HDLc/β	*P* value	Proximal gene	Ref.
1	rs2144300	228 361 539	8656	40	-	6.6×10^{-7}	*GALNT2*	[20]
	rs4846914	228 362 314	19 794	40	-0.05 SD	4.0×10^{-8}		[30]
1	rs4660293	39 800 767	> 100 000	23	-0.48	4.0×10^{-10}	*PABPC4*	[28]
1	rs1689800	180 435 508	> 100 000	35	-0.47	3.0×10^{-10}	*ZNF648*	[28]
2	rs1260326	27 584 444	16 682	41	0.93%	$< 5 \times 10^{-8}$	*GCKR*	[31]
2	rs6754295	21 059 688	17 915	25	2.63 (z-sc)[1]	4.4×10^{-8}	*APOB*	[27]
2	rs2972146	226 808 942	> 100 000	37	0.46	3.0×10^{-9}	*IRS1*	[28]
2	rs10490964	51 926 908	18 245	12	1.35 mg/dL	3.9×10^{-9}	*COBLL1, GRB14*	[26]
	rs12328675	165 249 046	> 100 000	13	0.68	3.0×10^{-10}	*COBLL1*	[28]
4	rs13107325	103 407 732	> 100 000	7	-0.84	7.0×10^{-11}	*SLC39A8*	[28]
5	rs6450176	53 333 782	> 100 000	26	-0.49	5.0×10^{-8}	*ARL15*	[28]
6	rs2814944	34 660 775	> 100 000	16	-0.49	4.0×10^{-9}	*C6orf106*	[28]
6	rs605066	139 871 359	> 100 000	42	-0.39	3.0×10^{-8}	*CITED2*	[28]
6	rs1084651	161 009 807	> 100 000	16	1.95	3.0×10^{-8}	*LPA*	[28]
7	rs4731702	130 083 924	> 100 000	48	0.59	1.0×10^{-15}	*KLF14*	[28]
8	rs2083637	19 909 455	17 922	26	4.14 (z-sc)[1]	5.5×10^{-18}	*LPL*	[27]
	rs10503669	19 891 970	8656	10		3.2×10^{-10}		[20]
	rs331	19 864 685	6382	28	1.5 mg/dL	9.1×10^{-7}		[32]
	rs17482753	19 876 926	8180	-		2.8×10^{-11}		[33]
	rs326	19 863 719	10 536	22-30		1.8×10^{-8}		[35]
	rs331	19 864 685	6382	28	1.5 mg/dL	9.1×10^{-7}		[32]
	rs12678919	19 888 502	19 794	10	0.23 SD	2.0×10^{-34}		[30]
	rs301	19 861 214	5592	25	0.04	9.3×10^{-11}		[36]
8	rs3916027	19 869 148	5592	27	0.04	5.4×10^{-10}	*SLC18A1*	[36]
8	rs331	19 864 685	16 809	27	0.43%	$< 5 \times 10^{-8}$	*Intergenic, PPP1R3B, LPL*	[31]
	rs9987289	9 220 768	> 100 000	9	-1.21	6.0×10^{-25}	*PPP1R3B*	[25]
8	rs2293889	116 668 374	> 100 000	0.41	-0.44	6.0×10^{-11}	*TRPS1*	[28]
9	r1323432	103 402 758	8656	12	1.93 mg/dL	2.5×10^{-8}	*GRIN3A*	[20]
9	rs3905000	106 696 891	17 913	14	-4.37 (z-sc)[1]	8.6×10^{-13}	*ABCA1*	[27]
	rs4149268	106 687 041	8656	36		3.3×10^{-7}		[20]
	rs3890182	106 687 476	21 312	-		3.0×10^{-10}		[29]
	rs9282541	106 660 656	10 536	0-9		4.8×10^{-8}		[35]
	rs2515614	106 724 139	16 798	34	0.20%	$< 5 \times 10^{-8}$		[31]
	rs1883025	106 704 122	19 371	26	-0.08 SD	1.0×10^{-9}		[30]
9	rs471364	15 279 578	40 414	12	-0.08 SD	3.0×10^{-10}	*TTC39B*	[29]
	rs581080	15 295 378	> 100 000	18	-0.65	3.0×10^{-12}		[28]
9	rs1883025	106 704 122	> 100 000	25	-0.94	2.0×10^{-33}	*ABCA1*	[28]
11	rs12225230	116 233 840	6382	18	1.5 mg/dL	5.3×10^{-5}	*APOA1/C3/A4/A5*	[32]
	rs618923	116 159 369	12 111	25	0.30%	$< 5 \times 10^{-8}$		[31]
	rs964184	116 154 127	19 794	14	-0.17 SD	1.0×10^{-12}		[30]
	rs7350481	116 091 493	8993	28	0.62%	8.8×10^{-10}		[36]
	rs7350481	116 091 493	18 245			2.8×10^{-12}		[26]
11	rs2923084	10 345 358	> 100 000	17	-0.41	5.0×10^{-8}	*AMPD3*	[28]
11	rs3136441	46 699 823	> 100 000	15	0.78	3.0×10^{-18}	*LRP4*	[28]
11	rs7395662		17 917	39	2.82 (z-sc)[1]	6.0×10^{-11}	*MADD-FOLH1*	[27]
11	rs174547	61 327 359	40 330	33	-0.09 SD	2.0×10^{-12}	*FADS1-S3*	[30]
11	rs6589565	116 145 447	5592	7	-0.05	4.4×10^{-7}	*BUD13*	[36]
11	rs2075290	116 158 506	5592	7	-0.05	4.2×10^{-7}	*ZNF259*	[36]
12	rs2338104	108 379 551	8656	45		1.9×10^{-6}	*MVK/MMAB*	[20]
	rs2338104	108 379 551	19 793	45	-0.07 SD	1.0×10^{-10}		[30]
	rs7134594	108 484 574	> 100 000	47	-0.44	7.0×10^{-15}		[28]
12	rs7134375	20 365 025	> 100 000	42	0.40	4.0×10^{-8}	*PDE3A*	[28]
12	rs4759375	122 362 191	> 100 000	6	0.86	7.0×10^{-9}	*SBNO1*	[28]
12	rs4765127	123 026 120	> 100 000	34	0.44	3.0×10^{-10}	*ZNF664*	[28]
12	rs838880	123 827 546	> 100 000	31	0.61	3.0×10^{-14}	*SCARB1*	[28]
12	rs1818702	102 047 685	16 844	29	0.22%	$< 5 \times 10^{-8}$	*Intergenic, ASCL1, PAH*	[31]
15	rs1532085	56 470 658	19 736	41	5.03 (z-sc)[1]	9.7×10^{-36}	*LIPC*	[27]
	rs4115041	121 186 681	8656	33		2.8×10^{-9}		[20]
	rs1532085	56 470 658	6382	37	1.8 mg/dL	1.3×10^{-10}		[32]
	rs1800588	56 510 967	21 312	-		2.0×10^{-32}		[29]
	rs11858164	56 530 023	10 536	27-55		7.0×10^{-8}		[35]
	rs1532085	56 470 658	6382	37	1.8 mg/dL	1.3×10^{-10}		[32]
	rs1800588	56 510 967	16 811	22	0.60%	$< 5 \times 10^{-8}$		[31]
	rs10468017	56 465 804	19 794	30	0.10 SD	8.0×10^{-23}		[30]
	rs1077834	56 510 771	5987	49	1.00%	1.3×10^{-14}		[36]

	rs1077834	56510771	18245			1.4×10^{-23}		[26]
	rs261342	56518445	5592	22	0.03	6.3×10^{-8}		[36]
	rs1532085	56470658	> 100000	39	1.45	3.0×10^{-96}		[28]
15	rs2652834	61183920	> 100000	20	-0.39	9.0×10^{-9}	*LACTB*	[28]
16	rs1800775	55552737	2623	47		2.5×10^{-13}	*CETP*	[28]
	rs1532624	55562980	19674	43	8.24 (z-sc)[1]	9.4×10^{-94}		[27]
	rs3764261	55550825	8656	31	2.42 mg/dL	2.8×10^{-19}		[20]
	rs3764261	55550825	6382	31	4.0 mg/dL	1.0×10^{-41}		[32]
	rs1800775	55552737	2758	49	2.6 mg/dL	3.0×10^{-13}		[29]
	rs1800775	55552737	1643	47	3.99 mg/dL	6.1×10^{-15}		[33]
	rs9989419	55542640	8216	-		8.5×10^{-27}		[33]
	rs7205804	55562390	10536	37-50		4.7×10^{-47}		[36]
	rs3764261	55550825	6382	31	4.0 mg/dL	1.0×10^{-41}		[32]
	rs1800775	55552737	16779	49	2.50%	$< 5 \times 10^{-8}$		[31]
	rs3764261	55550825	3228	-	6.2 mg/dL	3.4×10^{-12}		[39]
	rs3764261	55550825	18245			3.7×10^{-93}		[26]
	rs173539	55545545	19794	32	0.25 SD	4.0×10^{-75}		[30]
	rs3764261	55550825	5987	21	2.11%	4.8×10^{-29}		[37]
	rs3764261	55550825	18245	30-48		3.7×10^{-93}		[27]
	rs17231506	55552029	5592	32	0.07	2.3×10^{-36}		[36]
	rs3764261	55550825	> 100000	32	3.39	7.0×10^{-380}		[28]
16	rs255052	66582496	8656	17		1.5×10^{-6}	*LCAT*	[20]
	rs255052	66582496	8656 + 4534	-		1.2×10^{-7}		[20]
	rs2271293	66459571	31946	11	0.07 SD	9.0×10^{-13}		[30]
	rs16942887	66485543	> 100000	12	1.27	8.0×10^{-33}		[28]
16	rs2271293	66459571	17910	13	4.99 (z-sc)[1]	8.3×10^{-16}	*CTCF-PRMT8*	[27]
16	rs289743	55575297	5592	31	0.03	8.6×10^{-9}	*NLRC5*	[36]
16	rs2925979	80092291	> 100000	30	-0.45	2.0×10^{-11}	*CMIP*	[28]
17	rs11869286	35067382	> 100000	34	-0.48	1.0×10^{-13}	*STARD3*	[28]
17	rs4148008	64386889	> 100000	32	-0.42	2.0×10^{-10}	*ABCA8*	[28]
17	rs4129767	73915579	> 100000	49	-0.39	8.0×10^{-9}	*PGS1*	[28]
18	rs4939883	45421212	16258	17	-3.98 (z-sc)[1]	1.6×10^{-11}	*LIPG*	[27]
	rs2156552	45435666	8656	16		8.4×10^{-7}		[20]
	rs2156552	45435666	21312	-		2.0×10^{-7}		[29]
	rs4939883	45421212	16648	16	0.22%	$< 5 \times 10^{-8}$		[31]
	rs4939883	45421212	19785	17	-0.14 SD	7.0×10^{-15}		[30]
	rs4939883	45421212	18245			1.4×10^{-9}		[26]
	rs7241918	45414951	> 100000	17	-1.31	3.0×10^{-49}		[28]
18	rs12967135	56000003	> 100000	23	-0.42	7.0×10^{-9}	*MC4R*	[28]
19	rs769449	50101842	16728	12	0.30%	$< 5 \times 10^{-8}$	*APOC1-APOE*	[31]
			18245			2.6×10^{-11}		[26]
19	rs2967605	8375738	35151	16	-0.12 SD	1.0×10^{-8}	*ANGPTL3*	[30]
	rs7255436	8339196	> 100000	47	-0.45	3.0×10^{-8}		[28]
19	rs737337	11208493	> 100000	8	-0.64	3.0×10^{-9}	*LOC55908*	[28]
19	rs386000	59484573	> 100000	20	0.83	4.0×10^{-16}	*LILRA3*	[28]
20	rs6065906	43987422	16810	48	0.40%	$< 5 \times 10^{-8}$	*PLTP*	[31]
			18245			1.9×10^{-14}		[26]
20	rs1800961	42475778	30714	3	-0.19 SD	8.0×10^{-10}	*HNF4A*	[30]
	rs1800961	42475778	> 100000	3	-1.88	1.0×10^{-15}		[28]
20	rs7679	44009909	40248	19	-0.07 SD	4.0×10^{-9}	*PLTP*	[30]
	rs6065906	43987422	> 100000	18	-0.93	2.0×10^{-22}		[28]
22	rs181362	20262068	> 100000	20	-0.46	1.0×10^{-8}	*UBE2L3*	[28]

[1]z-sc: the ENGAGE consortium provided the effect size on the z-scale. GALNT2: N-acetylgalactosaminyltransferase 2; PABPC4: Poly(A) binding protein, cytoplasmic 4 (inducible form); ZNF648: Zinc finger protein 648; GCKR: Glucokinase (hexokinase 4) regulator; APOB: Apolipoprotein B; IRS1: Insulin receptor substrate 1; COBLL1: COBL-like 1; GRB14: Growth factor receptor-bound protein 14; SLC39A8: Solute carrier family 39 (zinc transporter) member 8; ARL15: ADP-ribosylation factor-like 15; C6orf106: Chromosome 6 open reading frame 106; CITED2: Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain 2; LPA: Lipoprotein, Lp(a); KLF14: Kruppel-like factor 14; LPL: Lipoprotein lipase; SLC18A1: Solute carrier family 18 (vesicular monoamine) member 1; PPP1R3B: Protein phosphatase 1, regulatory (inhibitor) subunit 3B; TRPS1: Trichorhinophalangeal syndrome 1; GRIN3A: Glutamate receptor, ionotropic, N-methyl-D-aspartate 3A; ABCA1: ATP-binding cassette, sub-family A (ABC1) member 1; TTC39B: Tetratricopeptide repeat domain 39B; ABCA1: ATP-binding cassette, sub-family A (ABC1) member 1; APOA1: Apolipoprotein A-I; AMPD3: Adenosine monophosphate deaminase 3; LRP4: Low density lipoprotein receptor-related protein 4; MADD-FOLH1: MAP-kinase activating death domain- folate hydrolase (prostate-specific membrane antigen) 1; FADS1-S3: Fatty acid desaturase 1; BUD13: BUD13 homolog; ZNF259: Zinc finger protein 259; MVK: Mevalonate kinase; MMAB: Methylmalonic aciduria (cobalamin deficiency) cblB type; PDE3A: Phosphodiesterase 3A; SBNO1: Strawberry notch homolog 1; ZNF664: Zinc finger protein 664; SCARB1: Scavenger receptor class B member 1; ASCL1: Achaete-scute complex homolog 1; PAH: Phenylalanine hydroxylase; LIPC: Hepatic lipase; LACTB: Lactamase β; CETP: Cholesteryl ester transfer protein plasma; LCAT: Lecithin-cholesterol acyltransferase; CTCF: CCCTC-binding factor (zinc finger protein); PRMT8: Protein arginine methyltransferase 8; NLRC5: NLR family CARD domain containing 5; STARD3: StAR-related lipid transfer (START) domain containing 3; ABCA8: ATP-binding cassette; sub-family A (ABC1) member 8; PGS1: Phosphatidylglycerophosphate synthase 1; LIPG: Lipase endothelial; MC4R: Melanocortin 4 receptor; APOC1: Apolipoprotein C-I; APOE: Apolipoprotein E; ANGPTL3: Angiopoietin-like 3; LILRA3: Leukocyte immunoglobulin-like receptor, subfamily A (without TM domain) member 3; PLTP: Phospholipid transfer protein; HNF4A: Hepatocyte nuclear factor 4 α; PLTP: Phospholipid transfer protein; UBE2L3: Ubiquitin-conjugating enzyme E2L 3; SNP: Single nucleotide polymorphisms; MAF: Minor allele frequency. HDLc: high-density lipoprotein cholesterol.

Table 4 Single nucleotide polymorphisms associated with low-density lipoprotein cholesterol identified through genome-wide association studies

Chromosome	Strongest SNP	Chromosome position	Sample size	MAF (average)	β	*P* value	Proximal gene	Ref.
1	rs1167998	62704220	12685	22	-0.155	7.8×10^{-23}	*PSRC1, CELSR2, SORT1*	[27]
	rs646776	109620053	6382	22	-	4.9×10^{-19}		[32]
	rs599839	109623689	1636	24	-	1.1×10^{-7}		[34]
	rs602633	109623034	8589	20	-	4.8×10^{-14}		[20]
	rs646776	109620053	16791	22	-0.04	$< 5 \times 10^{-8}$	*CELSR2*	[31]
	rs646776	109620053	21312	24	-0.16	5×10^{-42}	*PSRC1*	[29]
	rs12740374	109619113	19648	21	-0.23	2.0×10^{-42}		[30]
	rs599839	109623689	11685	21	-0.05	1.7×10^{-15}		[40]
	rs646776	109620053	4337	21	-0.16	4.3×10^{-9}		[40]
	rs12740374	109619113	5592	21	-0.15	1.8×10^{-9}	*SORT1*	[36]
	rs646776	109620053	5592	22	-0.14	3.8×10^{-8}		[36]
	rs629301	109619829	> 100000	22	-5.65	1.0×10^{-170}		[28]
1	rs11591147	55278235	16826	2	-0.12	$< 5 \times 10^{-8}$	*PCSK9*	[31]
	rs11591147	55278235	12167	2	-0.13	$< 5 \times 10^{-8}$		[31]
	rs11591147	55278235	21312	1	-0.26	2.0×10^{-24}		[30]
	rs11206510	55268627	19394			3.5×10^{-11}		[20]
	rs11206510	55268627	19629	19	-0.09	4.0×10^{-8}		[30]
	rs11591147	55278235	5592	2	-0.55	9.3×10^{-12}		[36]
	rs2479409	55277238	> 100000	30	2.01	2.0×10^{-28}		[28]
2	rs693	21085700	2601	22		7.1×10^{-7}	*APOB*	[38]
	rs562338	21141826	1636 + 2631	17		8.6×10^{-13}		[34]
	rs515135	21139562	8589	83		3.1×10^{-14}		[20]
	rs562338	21141826	8589 + 10849			5.6×10^{-22}		[20]
	rs693	21085700	16112	52	-0.098	3.6×10^{-17}		[28]
	rs506585	21250687	6382	20	-4.6	9.3×10^{-9}		[32]
	rs506585	21250687	16842	20	-0.04	$< 5 \times 10^{-8}$		[31]
	rs693	21085700	21312	48	0.12	1.0×10^{-21}		[29]
	rs515135	21139562	19648	20	-0.16	5.0×10^{-29}		[30]
	rs562338	21141826	11685	20	-0.04	1.4×10^{-9}		[40]
	rs1713222	21124828	4337	16	-0.17	1.0×10^{-8}		[40]
	rs562338	21141826	5592	18	-0.18	1.2×10^{-11}		[36]
	rs1367117	21117405	> 100000	30	4.05	4.0×10^{-114}		[28]
2	rs6756629	43918594	12706	92	0.157	2.6×10^{-10}	*ABCG5*	[27]
2	rs6544713	43927385	23456	32	0.15	2×10^{-20}	*ABCG8*	[30]
	rs4299376	43926080	> 100000	30	2.75	2.0×10^{-47}	*ABCG5/8*	[28]
2	rs780094	27594741	16841	40	0.03	$< 5 \times 10^{-8}$	*GCKR*	[31]
5	rs1501908	156330747	27280	37	-0.07	1×10^{-11}	*TIMD4-HAVCR1*	[30]
5	rs3846662	74686840	16135	44	0.079	1.5×10^{-11}	*HMGCR*	[27]
	rs12654264	74684359	2758 + 18554	39	0.1	1.0×10^{-20}		[29]
	rs3846663	74691482	19648	38	0.07	8.0×10^{-12}		[30]
	rs12654264	74684359	5592	38	0.11	5.8×10^{-8}		[36]
6	rs3757354	88570980	> 100000	22	-1.43	1.0×10^{-11}	*MYLIP*	[28]
6	rs1800562	26201120	> 100000	6	-2.22	6.0×10^{-10}	*HFE*	[28]
6	rs1564348	160498850	> 100000	17	-0.56	2.0×10^{-17}	*LPA*	[28]
7	rs12670798	21573877	12695	24	0.089	6.1×10^{-9}	*DNAH11*	[27]
7	rs4731702	130083924	16747	49	-0.02	$< 5 \times 10^{-8}$	*KLF14*	[31]
8	rs6982636	126548497	16798	47	-0.02	$< 5 \times 10^{-8}$	*TRIB1*	[31]
8	rs11136341	145115531	> 100000	40	1.4	4.0×10^{-13}	*PLEC1*	[28]
9	rs9411489	135144821	> 100000	20	2.24	6.0×10^{-13}	*ABO*	[28]
11	rs174570	61353788	16153	83	0.11	4.4×10^{-13}	*FADS2/3*	[31]
11	rs3135506	116167617	16837	6	-0.13	$< 5 \times 10^{-8}$	*APOA1-A5*	[31]
	rs2072560	116167036	5592	6	0.22	2.4×10^{-7}		[36]
11	rs11220462	125749162	> 100000	14	1.95	1.0×10^{-15}	*ST3GAL4*	[28]
12	rs7307277	123041109	16804	34	-0.02	$< 5 \times 10^{-8}$	*CCDC92/DNAH10/ZNF664*	[31]
12	rs2650000	119873345	39340	36	0.07	2.0×10^{-8}	*HNF1A*	[30]
14	rs8017377	51667587	> 100000	47	1.14	5.0×10^{-11}	*NYNRIN*	[28]
16	rs708272	55553789	16843	43	-0.04	$< 5 \times 10^{-8}$	*CETP*	[31]
	rs17231506	55552029	5592	32	-0.11	5.0×10^{-7}		[36]
17	rs7206971	42780114	> 100000	49	0.78	2.0×10^{-8}	*OSBPL7*	[28]
19	rs16996148	19519472	21312	10	-0.1	3×10^{-8}	*NCAN, CILP2, PBX4*	[29]
	rs2228603	19190924	8589	7		1.8×10^{-7}		[20]
	rs16996148	19519472	19394			2.7×10^{-9}		[20]
	rs10401969	19268718	19648	6	-0.05	2.0×10^{-8}		[30]
19	rs688	11088602	4267	45		7.3×10^{-7}	*LDLR*	[34]
	rs6511720	11063306	8589	9		6.8×10^{-10}		[20]

	rs6511720	11063306	19394			4.2×10^{-23}		[20]
	rs2228671	11071912	16148	82	0.136	4.2×10^{-14}		[27]
	rs6511720	11063306	6382	12	-7.7	5.2×10^{-15}		[32]
	rs6511720	11063306	16843	12	-0.04	$< 5 \times 10^{-8}$		[31]
	rs6511720	11063306	21312	10	-0.26	2×10^{-51}		[29]
	rs6511720	11063306	19648	10	-0.26	2.0×10^{-26}		[30]
	rs2228671	11071912	4337	12	-0.18	1.1×10^{-8}		[40]
	rs17248720	11059187	5592	13	-0.31	7.8×10^{-25}		[36]
	rs6511720	11063306	> 100000	11	6.99	4.0×10^{-117}		[28]
19	rs2075650	50087459	12697	15	0.16	9.3×10^{-19}	*TOMM40-APOE*	[27]
	rs157580	50087106	16160	68	-0.111	2.1×10^{-19}		[27]
	rs2075650	50087459	4337	13	0.23	7.1×10^{-14}		[40]
	rs2075650	50087459	5592	14	0.23	1.1×10^{-14}		[36]
19	rs4420638	50114786	2601	22		3.4×10^{-13}	*APOC1-APOE*	[38]
	rs4420638	50114786	4267	19		8.3×10^{-14}		[34]
	rs4420638	50114786	8589	12		1.5×10^{-21}		[20]
	rs4420638	50114786	19394			3.0×10^{-43}		[20]
	rs4803750	49939467	6382	7	-9.6	3.6×10^{-14}		[32]
	rs4803750	49939467	16616	7	-9.3	$< 5 \times 10^{-8}$		[32]
	rs4420638	50114786	21312	20	0.19	1.0×10^{-60}		[29]
	rs4420638	50114786	11881	16	0.29	4.0×10^{-27}		[30]
	rs4420638	50114786	11685	18	0.06	1.2×10^{-20}	*APOC2*	[40]
	rs12721046	50113094	5592	15	0.21	7.6×10^{-14}		[36]
	rs12721109	50139061	5592	2	-0.54	5.1×10^{-14}		[36]
	rs4420638	50114786	> 100000	17	7.14	9.0×10^{-147}	*APOE*	[28]
19	rs10402271	50021054	11685	33	0.03	4.1×10^{-8}	*BCAM*	[40]
	rs4605275	50030333	4337	31	-0.13	4.7×10^{-8}		[40]
19	rs4803750	49939467	4337	7	-0.28	2.4×10^{-11}	*BCL3*	[40]
	rs1531517	49934013	5592	7	-0.22	5.3×10^{-8}		[36]
19	rs10402271	50021054	5592	33	0.15	2.1×10^{-12}	*PVRL2*	[36]
20	rs6065906	43987422	16843	48	0.02	$< 5 \times 10^{-8}$	*PLPT*	[31]
20	rs6102059	38662198	28895	32	-0.06	4.0×10^{-9}	*MAFB*	[30]
20	rs6029526	39106032	> 100000	47	1.39	4.0×10^{-19}	*TOP1*	[28]

β: Estimated mean; CELSR2: Cadherin EGF LAG seven-pass G-type receptor 2; PSRC1: Proline/serine-rich coiled-coil 1; SORT1: Sortilin 1; PCSK9: Proprotein convertase subtilisin/kexin type 9; APOB: Apolipoprotein B; ABCG: ATP-binding cassette sub-family G; GCKR: Glucokinase (hexokinase 4) regulator; TIMD4: T-cell immunoglobulin and mucin domain containing 4; HAVCR1: Hepatitis A virus cellular receptor 1; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; MYLIP: Myosin regulatory light chain interacting protein; HFE: Human hemochromatosis; LPA: Lipoprotein, Lp(a); DNAH11: Dynein axonemal heavy chain 11; KLF14: Kruppel-like factor 14; TRIB1: Tribbles homolog 1; PLEC1: Plectin; ABO: ABO blood group (transferase A, α 1-3-N-acetylgalactosaminyltransferase, transferase B, α 1-3-galactosyltransferase); FADS: Fatty acid desaturase; APOA1: Apolipoprotein A1; ST3GAL4: ST3 β-galactoside α-2,3-sialyltransferase 4; CCDC92: Coiled-coil domain containing 92; DNAH10: Dynein axonemal heavy chain 10; ZNF664: Zinc finger protein 664; HNF1A: HNF1 homeobox A; NYNRIN: NYN domain and retroviral integrase containing; CETP: Cholesteryl ester transfer protein plasma; OSBPL7: Oxysterol binding protein-like 7; NCAN: Nucleoporin 214 kDa; CILP2: Cartilage intermediate layer protein 2; PBX4: Pre-B-cell leukemia homeobox 4; LDLR: Low density lipoprotein receptor; TOMM40: Translocase of outer mitochondrial membrane 40 homolog; APOE: Apolipoprotien E; APOC2: Apolipoprotein C-II; APOE: Apolipoprotein E; BCAM: Basal cell adhesion molecule; BCL3: B-cell CLL/lymphoma 3; PVRL2: Poliovirus receptor-related 2 (herpesvirus entry mediator B); PLPT: Proteolipid protein; MAFB: V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; TOP1: Topoisomerase (DNA) 1; SNP: Single nucleotide polymorphisms; MAF: Minor allele frequency.

CHD in the WTCCC study[17].

In total, 43 different loci have been found to be associated with triglycerides (TAG) in GWAS (Figure 5 and Table 5). SNPs in proximity to *ANGPTL3*, *APOB*, *GCKR*, *MLXIPL*, *LPL*, *TRIB1*, *APOA1/A4/A5/C3*, and *NCAN-CILP2-PBX4* have been associated with TAG in several GWAS.

GWAS AND BP

In 2007, the Framingham Heart Study[41] reported on 1327 individuals whose BP had been sampled longitudinally in the Framingham Community project. In the same year, the WTCCC[17] reported results from 2000 Northern European subjects with HTN. Although a few SNPs did reach a statistical significance of $P < 10^{-5}$, none of them achieved genome-wide significance ($P < 5 \times 10^{-8}$). The most significant GWAS findings in blood pressure are summarized in table 6 and figure 6[42-50].

The global BPgen consortium[42] studied 34433 subjects of European ancestry, subsequently followed up the findings with direct genotyping of 71225 individuals of European ancestry and 12889 individuals of Indian Asian ancestry and conducted a joint analysis. They identified an association between systolic or diastolic BP (SBP/DBP) and common variants in eight regions near the *CYP17A1* (intergenic *CNNM2/NT5C2*), *CYP1A2* (intron *CSK*), *FGF5*, *SH2B3* (intron *ATXN2*), *MTHFR*, c10orf107, *ZNF652* and intron *PLCD3*. Furthermore, three of these common variants (*MTHFR*, *CYP17A1* and *CYP17A2* or *CSK*) were associated with HTN ($P < 5 \times 10^{-8}$). The CHARGE consortium study ($n = 29136$)

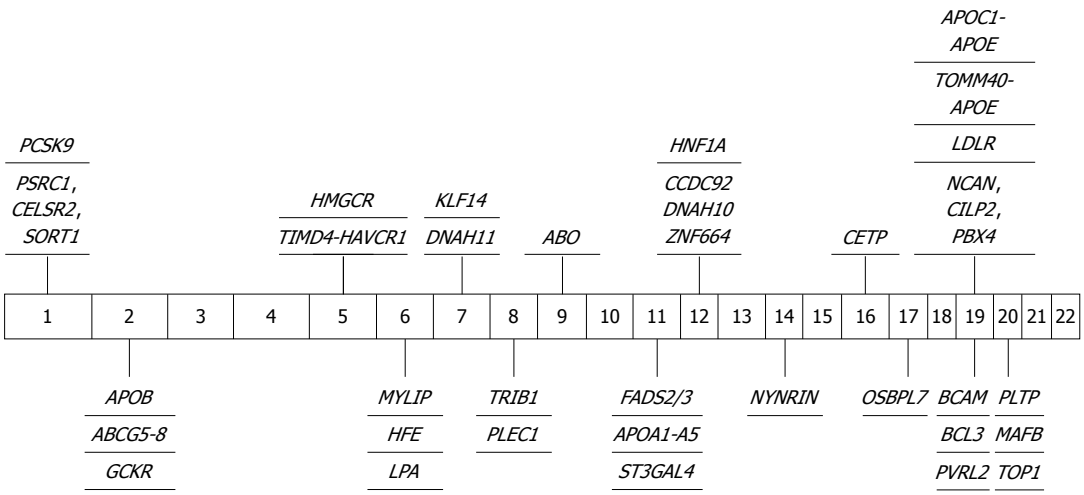

Figure 4 Significant genome-wide association study findings in low-density lipoprotein cholesterol. CELSR2: Cadherin EGF LAG seven-pass G-type receptor 2; PSRC1: Proline/serine-rich coiled-coil 1; SORT1: Sortilin 1; PCSK9: Proprotein convertase subtilisin/kexin type 9; APOB: Apolipoprotein B; ABCG: ATP-binding cassette sub-family G; GCKR: Glucokinase (hexokinase 4) regulator; TIMD4: T-cell immunoglobulin and mucin domain containing 4; HAVCR1: Hepatitis A virus cellular receptor 1; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; MYLIP: Myosin regulatory light chain interacting protein; HFE: Human hemochromatosis; LPA: Lipoprotein, Lp(a); DNAH11: Dynein axonemal heavy chain 11; KLF14: Kruppel-like factor 14; TRIB1: Tribbles homolog 1; PLEC1: Plectin; ABO: ABO blood group (transferase A, α 1-3-N-acetylgalactosaminyltransferase, transferase B, α 1-3-galactosyltransferase); FADS: Fatty acid desaturase; APOA1: Apolipoprotien A1; ST3GAL4: ST3 β-galactoside α-2;3-sialyltransferase 4; CCDC92: Coiled-coil domain containing 92; DNAH10: Dynein axonemal heavy chain 10; ZNF664: Zinc finger protein 664; HNF1A: HNF1 homeobox A; NYNRIN: NYN domain and retroviral integrase containing; CETP: Cholesteryl ester transfer protein plasma; OSBPL7: Oxysterol binding protein-like 7; NCAN: Nucleoporin 214kDa; CILP2: Cartilage intermediate layer protein 2; PBX4: Pre-B-cell leukemia homeobox 4; LDLR: Low-density lipoprotein receptor; TOMM40: Translocase of outer mitochondrial membrane 40 homolog; APOE: Apolipoprotein E; APOC2: Apolipoprotein C-II; BCAM: Basal cell adhesion molecule; BCL3: B-cell CLL/lymphoma 3; PVRL2: Poliovirus receptor-related 2 (herpesvirus entry mediator B); PLPT: Proteolipid protein; MAFB: V-maf musculoaponeurotic fibrosarcoma oncogene homolog B; TOP1: Topoisomerase (DNA) 1.

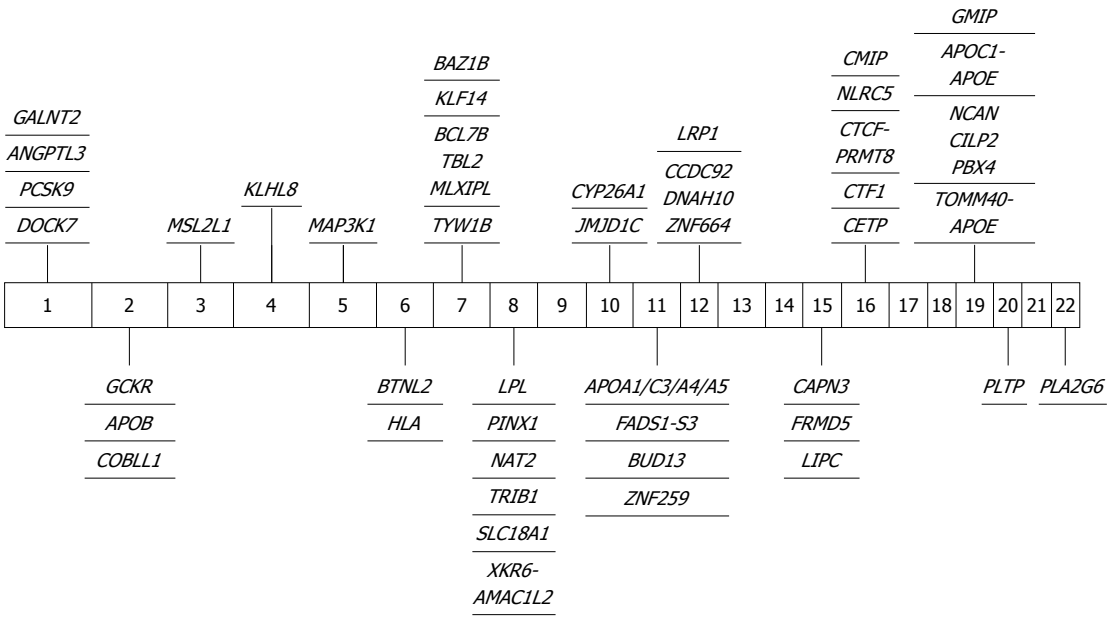

Figure 5 Significant genome-wide association study findings in triglycerides. DOCK7: Dedicator of cytokinesis 7; PCSK9: Proprotein convertase subtilisin/kexin type 9; GALNT2: N-acetylgalactosaminyltransferase 2; ANGPTL3: Angiopoietin-like 3; APOB: Apolipoprotien B; GCKR: Glucokinase (hexokinase 4) regulator; COBLL1: COBL-like 1; MSL2L1: Male-specific lethal 2 homolog; KLHL8: Kelch-like 8; MAP3K1: Mitogen-activated protein kinase kinase kinase 1; BTNL2: Butyrophilin-like 2 (MHC class II associated); HLA: Major histocompatibility complex; TYW1B: tRNA-yW synthesizing protein 1 homolog B; TBL2: Transducin (β)-like 2; BCL7B: B-cell CLL/lymphoma 7B; TBL2: Transducin (β)-like 2; MLXIPL: MLX interacting protein-like; KLF14: Kruppel-like factor 14; BAZ1B: Bromodomain adjacent to zinc finger domain 1B; PINX1: PIN2/TERF1 interacting, telomerase inhibitor 1; NAT2: N-acetyltransferase 2 (arylamine N-acetyltransferase); LPL: Lipoprotein lipase; PP1R3B: Protein phosphatase 1, regulatory (inhibitor) subunit 3B; TRIB1: Tribbles homolog 1; SLC18A1: Solute carrier family 18 (vesicular monoamine) member 1; XKR6: XK Kell blood group complex subunit-related family member 6; AMAC1L2: Acyl-malonyl condensing enzyme 1-like 2; JMJD1C: Jumonji domain containing 1C; CYP26A1: Cytochrome P450 family 26 subfamily A polypeptide 1; APOA1: Apolipoprotein A-I; FADS: Fatty acid desaturase; CCDC92: Coiled-coil domain containing 92; DNAH10: Dynein axonemal heavy chain 10; ZNF664: Zinc finger protein 664; LRP1: Low density lipoprotein receptor-related protein 1; CAPN3: Calpain 3, (p94); FRMD5: FERM domain containing 5; LIPC: Hepatic lipase; CTF1: Cardiotrophin 1; CETP: Cholesteryl ester transfer protein plasma; TOMM40: Translocase of outer mitochondrial membrane 40 homolog; APOE: Apolipoprotein E; NCAN: Nucleoporin 214kDa; CILP2: Cartilage intermediate layer protein 2; PBX4: Pre-B-cell leukemia homeobox 4; GMIP: GEM interacting protein; PLPT: Palmitoyl-protein thioesterase 1; PLA2G6: Phospholipase A2, group VI (cytosolic; calcium-independent).

Table 5 Single nucleotide polymorphisms associated with triglycerides identified through genome-wide association studies

Chromosome	Strongest SNP	Studies	Sample size	MAF (average)	β	*P* value	Proximal gene	Ref.
1	rs1167998	62704220	14268	32	-0.091	2.0×10^{-12}	*DOCK7*	[27]
	rs10889353	62890784	14337	32	-0.085	8.2×10^{-11}		[27]
1	rs11591147	55278235	16826	2	-0.09	$< 5 \times 10^{-8}$	*PCSK9*	[31]
1	rs12042319	62822407	4267	34		3.2×10^{-7}	*ANGPTL3*	[34]
	rs10889353	62890784	16831	33	-0.03	$< 5 \times 10^{-8}$		[31]
	rs10889353	62890784	8993	14	-0.13	2.0×10^{-9}		[37]
	rs12130333	62964365	21312	22	-0.11	2.0×10^{-8}		[29]
	rs10889353	62890784	19834	33	-0.05	3.0×10^{-7}		[30]
	rs1748195	62822181	18243			1.7×10^{-10}		[20]
	rs2131925	62798530	> 100000	32	-4.94	9.0×10^{-43}		[28]
1	rs4846914	228362314	21312	40	0.08	7.0×10^{-15}	*GALNT2*	[28]
2	rs6754295	21059688	14338	25	-0.077	2.5×10^{-8}	*APOB*	[27]
	rs673548	21091049	12694	76	0.086	1.1×10^{-8}		[27]
	rs673548	21091049	16797	21	-0.04	$< 5 \times 10^{-8}$		[31]
	rs693	21085700	21312	48	0.12	1.0×10^{-21}		[29]
	rs7557067	21061717	19840	22	-0.08	9.0×10^{-12}		[30]
	rs1042034	21078786	> 100000	22	-5.99	1.0×10^{-45}		[28]
2	rs780094	27594741	2659	35		3.7×10^{-8}	*GCKR*	[38]
	rs780094	27594741	4267	39		8.1×10^{-14}		[34]
	rs1260326	27584444	8684	40		1.5×10^{-15}		[20]
	rs780094	27594741	18243			6.1×10^{-32}		[20]
	rs780094	27594741	17790	63	-0.103	3.1×10^{-20}		[27]
	rs1260326	27584444	6382	41	0.07	1.3×10^{-16}		[32]
	rs1260326	27584444	16650	41	0.07	$< 5 \times 10^{-8}$		[31]
	rs1260326	27584444	8993	45	-0.101	1.1×10^{-11}		[37]
	rs780094	27594741	21312	34	0.13	3.0×10^{-14}		[29]
	rs1260326	27584444	19840	45	0.12	2.0×10^{-31}		[30]
	rs1260326	27584444	5592	40	0.06	1.8×10^{-7}		[36]
	rs1260326	27584444	> 100000	41	8.76	6.0×10^{-133}		[28]
2	rs10195252	165221337	> 100000	40	-2.01	2.0×10^{-10}	*COBLL1*	[28]
3	rs645040	137409312	> 100000	22	-2.22	3.0×10^{-8}	*MSL2L1*	[28]
4	rs442177	88249285	> 100000	41	-2.25	9.0×10^{-12}	*KLHL8*	[28]
5	rs9686661	55897543	> 100000	20	2.57	1.0×10^{-10}	*MAP3K1*	[28]
6	rs2076530	32471794	16829	43	0.03	$< 5 \times 10^{-8}$	*BTNL2*	[31]
6	rs2247056	31373469	> 100000	25	-2.99	2.0×10^{-15}	*HLA*	[28]
7	rs13238203	71767603	> 100000	4	-7.91	1.0×10^{-9}	*TYW1B*	[28]
7	rs17145738	72620810	2758 + 18554	13	-0.14	7.0×10^{-22}	*BCL7B, TBL2, MLXIPL TBL2*	[29]
	rs11974409	72627326	5592	20	-0.08	5.7×10^{-9}	*MLXIPL*	[36]
	rs10551921	107998852	5592	20	-0.08	1.3×10^{-8}		[36]
7	rs2240466	72494205	12680	87	0.137	1.1×10^{-12}	*MLXIPL*	[27]
	rs11974409	72627326	16839	19	-0.04	$< 5 \times 10^{-8}$		[31]
	rs714052	72502805	19840	12	-0.16	3.0×10^{-15}		[30]
	rs17145738	72620810	18243			2.0×10^{-12}		[20]
	rs17145738	72620810	> 100000	12	-9.32	6.0×10^{-58}		[28]
7	rs4731702	130083924	16714	49	-0.03	$< 5 \times 10^{-8}$	*KLF14*	[31]
7	rs17145713	72542746	5592	20	-0.09	5.3×10^{-10}	*BAZ1B*	[36]
8	rs11776767	10721339	> 100000	37	2.01	1.0×10^{-8}	*PINX1*	[28]
8	rs1495741	18317161	> 100000	22	2.85	5.0×10^{-14}	*NAT2*	[28]
8	rs2083637	19909455	14344	26	-0.107	1.0×10^{-14}	*LPL*	[27]
	rs10096633	19875201	12708	88	0.174	1.9×10^{-18}		[27]
	rs12678919	19888502	19840	10	-0.25	2.0×10^{-41}		[30]
	rs10096633	19875201	8993	12	-0.169	9.3×10^{-14}		[37]
	rs331	19864685	5592	25	-0.08	1.7×10^{-11}		[36]
	rs12678919	19888502	> 100000	12	-13.64	2.0×10^{-115}		[28]
8	rs17482753	19876926	2652	11		4.9×10^{-7}	*LPL*	[38]
	rs17482753	19876926	1636	10		1.2×10^{-9}		[34]
	rs17482753	19876926	1636 + 2631			5.2×10^{-15}		[34]
	rs6993414	19947198	8684	46		1.4×10^{-13}		[20]
	rs10503669	19891970	4267			3.9×10^{-22}		[20]
	rs328	19864004	6382	11	-0.09	4.7×10^{-11}	*Intergenic, PPP1R3B, LPL*	[32]
	rs331	19864685	6382	28	-0.06	1.7×10^{-9}		[32]
	rs328	19864685	16812	11	-0.09	$< 5 \times 10^{-8}$	*LPL*	[31]
	rs328	19864004	21242	9	-0.19	2.0×10^{-28}		[29]
8	rs6982636	126548497	16765	47	-0.03	$< 5 \times 10^{-8}$	*TRIB1*	[31]
	rs17321515	12655591	21242	49	-0.08	4.0×10^{-17}		[29]
	rs2954029	126560154	8684	56		2.8×10^{-8}		[20]

	rs17321515	12655591	14176			7.0×10^{-13}		[20]
	rs2954029	126560154	19840	44	-0.11	3.0×10^{-19}		[30]
	rs2954029	126560154	> 100000	47	-5.64	3.0×10^{-55}		[28]
8	rs3916027	19869148	5592	27	-0.08	1.0×10^{-10}	*SLC18A1*	[36]
8	rs7819412	11082571	33336	48	-0.04	3.0×10^{-8}	*XKR6-AMAC1L2*	[30]
10	rs10761731	64697616	> 100000	43	-2.38	3.0×10^{-12}	*JMJD1C*	[28]
10	rs2068888	94829632	> 100000	46	-2.28	2.0×10^{-8}	*CYP26A1*	[28]
11	rs12272004	116108934	12622	7	-0.181	5.4×10^{-13}	*APO (A1/A4/A5/C3)*	[27]
	rs6589566	116157633	1636	6		1.5×10^{-11}		[34]
	rs6589566	116157633	1636 + 2631			3.7×10^{-12}		[34]
	rs964184	116154127	8684	12		1.5×10^{-16}		[20]
	rs12286037	116157417	18422			1.0×10^{-26}		[20]
	rs3135506	116167617	6382	6	0.13	5.5×10^{-12}		[32]
	rs662799	116168917	6382	6	0.14	2.9×10^{-15}		[32]
	rs3135506	116167617	16804	6	0.14	$< 5 \times 10^{-8}$		[31]
	rs7350481	116091493	8993	43	0.24	1.4×10^{-49}		[37]
	rs28927680	116124283	21312	7	0.26	2.0×10^{-17}		[29]
	rs964184	116154127	19840	14	0.3	4.0×10^{-62}		[30]
	rs651821	116167789	5592	6	0.21	8.8×10^{-21}	*APOA1*	[36]
	rs964184	116154127	> 100000	13	16.95	7.0×10^{-240}		[28]
11	rs174547	61327359	38846	33	0.06	2.0×10^{-14}	*FADS1-S3*	[30]
	rs174546	61326406	> 100000	34	3.82	5.0×10^{-24}		[28]
11	rs6589565	116145447	5592	7	0.19	4.5×10^{-20}	*BUD13*	[36]
11	rs2075290	116158506	5592	7	0.19	6.6×10^{-20}	*ZNF259*	[36]
12	rs7307277	123041109	16771	34	-0.04	$< 5 \times 10^{-8}$	*CCDC92/DNAH10/ ZNF664*	[31]
12	rs11613352	-	> 100000	23	-2.7	4.0×10^{-10}	*LRP1*	[28]
15	rs2412710	40471079	> 100000	2	7	2.0×10^{-8}	*CAPN3*	[28]
15	rs2929282	42033223	> 100000	5	5.13	2.0×10^{-11}	*FRMD5*	[28]
15	rs4775041	56461987	8684	67		7.3×10^{-5}	*LIPC*	[20]
	rs4775041	56461987	17104			1.6×10^{-8}		[20]
16	Rs11649653	30825988	> 100000	40	-2.13	3.0×10^{-8}	*CTF1*	[28]
16	rs1800775	55552737	16779	49	-0.03	$< 5 \times 10^{-8}$	*CETP*	[31]
19	rs157580	50087106	16160	33	-0.069	1.2×10^{-8}	*TOMM40-APOE*	[27]
	rs439401	50106291	11885	68	0.086	1.8×10^{-9}		[27]
19	rs16996148	19519472	21312	10	-0.1	4.0×10^{-9}	*NCAN, CILP2, PBX4*	[29]
	rs10401969	19268718	8684	8		2.3×10^{-7}		[20]
	rs16996148	19519472	18391			2.5×10^{-9}		[20]
	rs17216525	46471516	19840	7	-0.11	4.0×10^{-11}		[30]
	rs12610185	19582722	5592	9	-0.1	5.6×10^{-7}		[36]
19	rs439401	50106291	16638	35	-0.04	$< 5 \times 10^{-8}$	*APOC1-APOE*	[31]
	rs439401	50106291	> 100000	36	-5.5	1.0×10^{-30}	*APOE*	[28]
19	rs2304128	19607151	5592	9	-0.1	3.2×10^{-7}	*GMIP*	[36]
20	rs6065906	43987422	16810	48	0.04	$< 5 \times 10^{-8}$	*PLPT*	[31]
	rs7679	44009909	38561	19	0.07	7.0×10^{-11}		[30]
22	rs5756931	36875979	> 100000	40	-1.54	4.0×10^{-8}	*PLA2G6*	[28]

β: Estimated mean; DOCK7: Dedicator of cytokinesis 7; PCSK9: Proprotein convertase subtilisin/kexin type 9; GALNT2: N-acetylgalactosaminyltransferase 2; ANGPTL3: Angiopoietin-like 3; APOB: Apolipoprotein B; GCKR: Glucokinase (hexokinase 4) regulator; COBLL1: COBL-like 1; MSL2L1: Male-specific lethal 2 homolog; KLHL8: Kelch-like 8; MAP3K1: Mitogen-activated protein kinase kinase kinase 1; BTNL2: Butyrophilin-like 2 (MHC class II associated); HLA: Major histocompatibility complex; TYW1B: tRNA-yW synthesizing protein 1 homolog B; TBL2: Transducin (β)-like 2; BCL7B: B-cell CLL/lymphoma 7B; TBL2: Transducin (β)-like 2; MLXIPL: MLX interacting protein-like; KLF14: Kruppel-like factor 14; BAZ1B: Bromodomain adjacent to zinc finger domain 1B; PINX1: PIN2/TERF1 interacting, telomerase inhibitor 1; NAT2: N-acetyltransferase 2 (arylamine N-acetyltransferase); LPL: Lipoprotein lipase; PP1R3B: Protein phosphatase 1, regulatory (inhibitor) subunit 3B; TRIB1: Tribbles homolog 1; SLC18A1: Solute carrier family 18 (vesicular monoamine) member 1; XKR6: XK Kell blood group complex subunit-related family member 6; AMAC1L2: Acyl-malonyl condensing enzyme 1-like 2; JMJD1C: Jumonji domain containing 1C; CYP26A1: Cytochrome P450 family 26 subfamily A polypeptide 1; APOA1: Apolipoprotein A-I; FADS: Fatty acid desaturase; CCDC92: Coiled-coil domain containing 92; DNAH10: Dynein axonemal heavy chain 10; ZNF664: Zinc finger protein 664; LRP1: Low density lipoprotein receptor-related protein 1; CAPN3: Calpain 3, (p94); FRMD5: FERM domain containing 5; LIPC: Hepatic lipase; CTF1: Cardiotrophin 1; CETP: Cholesteryl ester transfer protein plasma; TOMM40: Translocase of outer mitochondrial membrane 40 homolog; APOE: Apolipoprotein E; NCAN: Nucleoporin 214 kDa; CILP2: Cartilage intermediate layer protein 2; PBX4: Pre-B-cell leukemia homeobox 4; GMIP: GEM interacting protein; PLPT: Palmitoyl-protein thioesterase 1; PLA2G6: Phospholipase A2, group VI (cytosolic, calcium-independent); SNP: Single nucleotide polymorphisms; MAF: Minor allele frequency.

identified 13, 20 and 10 SNPs for SBP, DBP and HTN respectively[43].

In a joint meta-analysis of CHARGE consortium data with BPgen consortium data (n = 34433)[43], four CHARGE loci attained genome-wide significance for SBP (*ATP2B1*, *CYP17A1*, *PLEKHA7*, *SH2B3*), six for DBP (*ATP2B1*, *CACNB2*, *CSK-ULK3*, *SH2B3*, *TBX3-TBX5*, *ULK4*) and one for HTN (*ATP2B1*). The KORA study by Org *et al*[48] in a South German Cohort identified a SNP upstream of T-cadherin adhesion molecule (*CDH13*) gene on chromosome 16 (rs11646213) as significantly associated with HTN at a genome-wide level. Finally, in a

Table 6 Single nucleotide polymorphisms associated with hypertension and blood pressure in genome-wide association studies

Chr	SNP	Position	Ancestry	N (discovery)	Phenotype	Risk allele	Risk allele frequency	OR/β	*P*	Nearest gene	Ref.
1	rs17367504	11785365	E	34433	SBP	G	0.14	-0.85	2×10^{-13}	*MTHFR, CLCN6, NPPA, NPPB, AGTRAP*	[42,43]
2	rs6749447	168749632	E	542	SBP	G	0.28	1.90	8×10^{-5}	*STK39*	[47]
3	rs9815354	41887655	E	29136	DBP	A	0.17	0.49	3×10^{-9}	*ULK4*	[42,43]
4	rs16998073	81403365	E	34433	DBP	T	0.21	0.50	1×10^{-21}	*FGF5, PRDM8, C4orf22*	[42,43]
4	rs991316	100541468	AA	1017	SBP	T	0.45	1.62	5×10^{-6}	*ADH7*	[44]
10	rs11014166	18748804	E	29136	DBP	A	0.66	0.37	1×10^{-8}	*CACNB2*	[42,43]
10	rs1530440	63194597	E	34433	DBP	T	0.19	-0.39	1×10^{-9}	*C10orf107, TMEM26, RTKN2, RHOBTB1, ARID5B, CYP17A1*	[42,43]
10	rs1004467	104584497	E	29136	SBP	A	0.90	1.05	1×10^{-10}	*TMEM26, RTKN2, RHOBTB1, ARID5B, CYP17A1*	[42,43]
10	rs11191548	104836168	E	34433	SBP	T	0.91	1.16	3×10^{-7}	*CYP17A1, AS3MT, CNNM2, NT5C2*	[42,43]
11	rs381815	16858844	E	29136	SBP	T	0.26	0.65	2×10^{-9}	*PLEKHA7*	[42,43]
12	rs17249754	88584	EA	8842	SBP, DBP	A	0.37	1.06	9×10^{-7}	*ATP2B1*	[49]
12	rs2681472	88533090	E	29136	SBP, DBP, HTN	A	0.83	0.50	2×10^{-9}	*ATP2B1*	[42,43]
12	rs2681492,	88537220	E	29136	SBP, DBP, HTN	T	0.80	0.85	4×10^{-11}	*ATP2B1*	[42,43]
12	rs3184504	110368991	E	29136	SBP, DBP	T	0.49	0.48	3×10^{-14}	*ATXN2, SH2B3*	[42,43]
12	rs653178	110492139	E	34433	DBP	T	0.53	-0.46	3×10^{-18}	*ATXN2, SH2B3*	[42,43]
12	rs2384550	113837114	E	29136	DBP	A	0.35	0.43	4×10^{-8}	*TBX3, TBX5*	[42,43]
15	rs1550576	56000706	AA	1017	SBP	C	0.86	1.92	3×10^{-6}	*ALDH1A2*	[44]
15	rs1378942	72865396	E	34433	DBP	C	0.36	0.43	1×10^{-23}	*CSK, CYP1A1, CYP1A2, LMAN1L, CPLX3, ARID3B, ULK3*	[42,43]
15	rs6495122	72912698	E	29136	DBP	A	0.42	0.40	2×10^{-10}	*CSK, CYP1A1, CYP1A2, LMAN1L, CPLX3, ARID3B, ULK3*	[42,43]
16	rs13333226	20273155	E	3320	HTN	A	0.81	1.15	4×10^{-11}	*UMOD*	[50]
16	rs11646213	81200152	E	1977	HTN	T	0.60	1.28	8×10^{-6}	*CDH13*	[48]
17	rs12946454	40563647	E	34433	SBP	T	0.28	0.57	1×10^{-8}	*PLCD3, ACBD4, HEXIM1, HEXIM2*	[42,43]
17	rs16948048	44795465	E	34433	DBP	G	0.39	0.31	5×10^{-9}	*ZNF652, PHB*	[42,43]

E: European; AA: African American; EA: East Asians; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HTN: Hypertension; ACBD4: Acyl-CoA binding domain containing 4; ADH7: Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide; AGTRAP: Angiotensin II receptor-associated protein; ALDH1A2: Aldehyde dehydrogenase 1 family, member A2; ARID5B: AT rich interactive domain 5B (MRF1-like); AS3MT: Arsenic (+3 oxidation state) methyltransferase; ATP2B1: ATPase; Ca^{++} transporting; plasma membrane 1; ATXN2: Ataxin 2; C10orf107: Chromosome 10 open reading frame 107; C4orf22: Chromosome 4 open reading frame 22; CACNB2: Calcium channel, voltage-dependent, β 2 subunit; CDH13: Cadherin 13, H-cadherin (heart); CLCN6: Chloride channel 6; CNNM2: Cyclin M2; CPLX3: Complexin 3; CSK: C-src tyrosine kinase; CYP17A1: Cytochrome P450, family 17, subfamily A, polypeptide 1; CYP1A1: Cytochrome P450; family 1, subfamily A, polypeptide 1; CYP1A2: Cytochrome P450, family 1, subfamily A, polypeptide 2; FGF5: Fibroblast growth factor 5; HEXIM1: Hexamethylene bis-acetamide inducible 1; HEXIM2: Hexamthylene bis-acetamide inducible 2; LMAN1L: Lectin, mannose-binding, 1 like; MTHFR: Methylenetetrahydrofolate reductase (NAD(P)H); NPPA: Natriuretic peptide A; NPPB: Natriuretic peptide B; NT5C2: 5'-nucleotidase, cytosolic II; PHB: Prohibitin; PLCD3: Phospholipase C, Δ 3; PLEKHA7: Pleckstrin homology domain containing, family A member 7; PRDM8: PR domain containing 8; RHOBTB1: Rho-related BTB domain containing 1; RTKN2: Rhotekin 2; SH2B3: SH2B adaptor protein 3; STK39: Serine threonine kinase 39; TBX3: T-box 3; TBX5: T-box 5; TMEM26: Transmembrane protein 26; ULK3: Unc-51-like kinase 3 (C. elegans); ULK4: Unc-51-like kinase 4 (C. elegans); UMOD: Uromodulin, ZNF652: Zinc finger protein 652; SNP: Single nucleotide polymorphisms; OR: Odds ratio.

population of African origin, Adeyemo *et al*[44] identified four common variants (*MYLIP*, chr 6; *YWHAZ*, chr 8; *IPO7*, chr 11 and *SLC24A4*, chr 14) associated with SBP with genome-wide significance.

Wang *et al*[47] identified *STK39*, *SPAK* (STE20/SPS1-related proline and alanine rich kinase; a serine/threonine kinase) with a *P* value of 1.6×10^{-7} in an Amish cohort. Several other studies also identified potentially important genetic loci associated with BP traits with borderline genome-wide significance. These include *ATP2B1*[43,51] (ATPase, Ca^{++} transporting, plasma membrane 1) on chromosome 12, *FOXD3*[41] (fork head box D3) on chromosome 1, *CCNG1* (cyclin G1)[48] on chromosome 5, *BCAT1* (branched chain aminotransferase 1, cytosolic)[17] on chromosome 12, *ATXN2* (ataxin 2)[42,43] on chromosome 12 and *TBX3* (T-box3)[43] on chromosome 12 (Figure 6

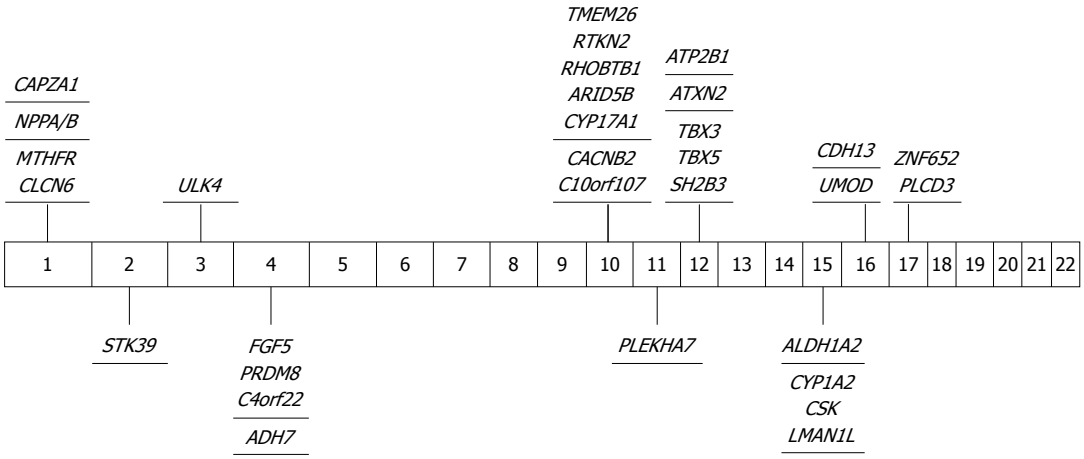

Figure 6 Significant genome-wide association study findings in blood pressure. ACBD4: Acyl-CoA binding domain containing 4; ADH7: Alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide; AGTRAP: Angiotensin II receptor-associated protein; ALDH1A2: Aldehyde dehydrogenase 1 family, member A2; ARID5B: AT rich interactive domain 5B (MRF1-like); AS3MT: Arsenic (+3 oxidation state) methyltransferase; ATP2B1: ATPase, Ca^{++} transporting; plasma membrane 1; ATXN2: Ataxin 2; C10orf107: Chromosome 10 open reading frame 107; C4orf22: Chromosome 4 open reading frame 22; CACNB2: Calcium channel, voltage-dependent, β 2 subunit; CDH13: Cadherin 13, H-cadherin (heart); CLCN6: Chloride channel 6; CNNM2: Cyclin M2; CPLX3: Complexin 3; CSK: C-src tyrosine kinase; CYP17A1: Cytochrome P450, family 17, subfamily A; polypeptide 1; CYP1A1: Cytochrome P450, family 1, subfamily A, polypeptide 1; CYP1A2: Cytochrome P450, family 1, subfamily A, polypeptide 2; FGF5: Fibroblast growth factor 5; HEXIM1: Hexamethylene bis-acetamide inducible 1; HEXIM2: Hexamthylene bis-acetamide inducible 2; LMAN1L: Lectin, mannose-binding, 1 like; MTHFR: Methylenetetrahydrofolate reductase (NAD(P)H); NPPA: Natriuretic peptide A; NPPB: Natriuretic peptide B; NT5C2: 5'-nucleotidase, cytosolic II; PHB: Prohibitin; PLCD3: Phospholipase C, Δ 3; PLEKHA7: Pleckstrin homology domain containing, family A member 7; PRDM8: PR domain containing 8; RHOBTB1: Rho-related BTB domain containing 1; RTKN2: Rhotekin 2; SH2B3: SH2B adaptor protein 3; STK39: Serine threonine kinase 39; TBX3: T-box 3; TBX5: T-box 5; TMEM26: Transmembrane protein 26; ULK3: Unc-51-like kinase 3 (C. elegans); ULK4: Unc-51-like kinase 4 (C. elegans); UMOD: Uromodulin; ZNF652: Zinc finger protein 652.

and Table 6). However, none of these loci were replicated in other studies. Using an extreme case-control design, Padmanabhan *et al*[50] identified a novel HTN locus on chromosome 16 in the promoter region of uromodulin (UMOD; rs13333226, combined P value 3.6×10^{-11}). The minor G allele of this SNP is associated with a lower risk of HTN [OR (95% CI): 0.87 (0.84-0.91)], reduced urinary UMOD excretion and increased estimated glomerular filtration rate (3.6 mL/min per minor-allele, $P = 0.012$), and borderline association with renal sodium balance.

CLINICAL IMPLICATIONS

GWAS are a useful tool in the identification of new and unexpected genetic loci of common diseases and traits, thus providing key novel insights into disease biology. But the clinical utility of these discoveries is negligible at this stage. The comparatively small numbers of variants which have been successfully replicated in several independent studies explain only a small proportion of the observed variation of these traits and explain in aggregate less than 20% of disease heritability. For example, the loci underpinning LDL-C levels[28] and BP account for < 20% of the variance of these quantitative traits. The variants associated with CHD increase disease risk by up to 20% per allele[51,52]. Next generation sequencing is now used to study low-frequency and rare variants that may potentially explain some of the missing heritabilities; however it is likely that studies designed to test for gene-environment interactions and gene-gene interactions may hold the answer. There were attempts to develop genetic profiles using the results from GWAS studies, but these have very limited value in personalised risk prediction as the genotype-phenotype effect sizes are very small. In the few studies that have evaluated the ability of a panel of genetic markers to discriminate CHD cases, the area under the receiver operating characteristic curve has been small indicating that conventional risk factors and family history are better at predicting risk and the incremental advantage of adding genetic markers is negligible. A few studies have attempted reclassification based on incorporation of SNPs from GWAS of CAD, lipids, *etc*.[52-58], and while they showed some improvement in net reclassification, the interpretation of these are still controversial and not translatable into general use[59]. Many companies are providing direct-to-consumer genetic tests that provide a "genetic risk profile" for an individual using risk alleles of small-to-moderate effects despite the clinical utility of genetic screening not being established. None of the major healthcare providers in Europe and USA have adopted these tests for CHD risk prediction, and the FDA has advised that direct-to-consumer genetic tests should be considered to be medical devices requiring FDA approval for commercial use. The future application of genetic screening will be in identifying risk groups early in life to direct targeted preventive measures and potentially pharmacogenetic tests to identify those at higher risk for adverse events. While technology is not a barrier to achieving this, the discovery, evaluation and deployment of these tests will require the same standards as non-genetic tests[60].

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REVIEW

Phase I of cardiac rehabilitation: A new challenge for evidence based physiotherapy

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Abstract

Cardiac rehabilitation protocols applied during the in-hospital phase (phase I) are subjective and their results are contested when evaluated considering what should be the three basic principles of exercise prescription: specificity, overload and reversibility. In this review, we focus on the problems associated with the models of exercise prescription applied at this early stage in-hospital and adopted today, especially the lack of clinical studies demonstrating its effectiveness. Moreover, we present the concept of "periodization" as a useful tool in the search for better results.

Key words: Cardiac rehabilitation; Exercise physiology; Physical therapy

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INTRODUCTION

There are different levels of physical aptitude, and they are directly related to the functional capacity of each individual. The same person presents different levels of physical aptitude in different phases of one's life. Physical aptitude is determined by two different components: musculoskeletal and cardiopulmonary. The improvement of both is achieved with regular training (physical activity) and is associated with better exercise tolerance. On the other hand, physical aptitude can deteriorate due to disuse[1]. Bed rest significantly decreases the cardiovascular tolerance for exercise in normal subjects and contributes to physical debility[2-4].

The main components of physical aptitude include body composition, cardio-respiratory aptitude, muscle strength and flexibility[5]. All of them are directly compromised by bed restriction. The human body works like a fine-tuned gear system involving the respiratory, cardiovascular and musculoskeletal systems[6]. The first

is responsible for capturing atmospheric oxygen and distributing it into the blood stream through diffusion. The second is responsible for the systemic distribution of oxygenated blood which depends on the pumping function of the left heart. The third system (musculoskeletal) is responsible for capturing and extracting molecular oxygen from the blood stream and transforming it into energy through intracellular biochemical reactions, in order to generate movement[7].

The whole system improves as the individual exercises and the organism develops physiological adaptations[8]. In many individuals one or more of these gears can be pathologically compromised either by clinical disease, surgical intervention and/or by being bedridden. Patients with respiratory diseases tend to have difficulty in capturing and diffusing oxygen, as a result of alterations in their pulmonary volumes and capacities, interfering directly in their exercise tolerance[9]. Patients with decreased left ventricular function present a reduction in the ejection fraction and decrease in the amount of systemic oxygen, consequently reducing exercise tolerance[10,11].

When cardiopathies and pneumopathies coexist in the same patient, the results of such physiopathological alterations can be a reduction in muscle strength due to disuse induced by lower tolerance to exercise or due to the lower level of physical aptitude[12,13]. When such a population is submitted to an exercise program, psychopathological adaptations of the organism are observed[14], and specific methods of exercise prescription are necessary to fulfill the needs of these patients[15], taking into account each personal and functional adaptation[16].

CLINICAL EXERCISE PHYSIOLOGY IN HOSPITAL PHASE

Clinical exercise physiology is still an incipient science, as evidenced by the weakness of the exercise prescription models for the hospital phase. At this early phase, the focus of physical therapy is prescription to avoid inactivity, and to maintain or improve pulmonary capacities and muscular strength[1,17,18], especially in patients after cardiac surgery. Although it might seem simple, many authors question the efficacy of these physical therapy programs. Sivarajan *et al*[19] evaluated a type of in-hospital exercise prescription in patients who suffered a myocardial infarction and found no improvement in the treadmill performance. There is a low level of evidence to assess the efficacy of chest physical therapy in the hospital phase.

EFFICACY OF CHEST PHYSICAL THERAPY APPLIED IN POST CARDIAC SURGERY

A systematic review was carried out to determine the efficacy of the use of physiotherapy in the prevention of pulmonary complications in post-operative heart surgery patients and to assess the efficacy of chest physical therapy in the hospital phase[20]. Eighteen trials, involving 1457 patients were evaluated: 13 evaluated the use of respiratory physiotherapy in post-operative heart surgery patients; 8 evaluated respiratory boosters; 5 tested the efficacy of applying a continuous positive airway pressure system; and 3 tested the efficacy of applying intermittent positive pressure. Changes in the following variables were assessed: incidence of atelectasis and pneumonia; partial oxygen pressure in the arterial blood; forced vital capacity; and forced expiratory volume in the first second. No intervention showed superiority for any end point confirming findings from previous studies[21,22]. However, it is well accepted that patients submitted to cardiac or thoracic surgery need ventilatory special attention.

As a result of sternotomy and the insertion of thoracic and mediastinum drainage tubes, the majority of these patients tend to present a restrictive disturbance with reductions in the pulmonary volumes and capacities[23]. Thus ventilation tends to be compromised and the diffusion of systemic oxygen also tends to be reduced[24], as shown by the low oxygen levels found in the arterial blood in exams such as gasometry, up to 72 h after the surgical procedure. The exercise tolerance of these patients tends to be lower, demanding an exercise prescription that respects the balance between oxygen offer and consumption, avoiding an overload which, in this specific case, can lead to hemodynamic instability[25-27]. So there is a clear paradox: although it seems clearly reasonable to accept that respiratory interventions are needed in this population during phase I of cardiac rehabilitation, this is still not supported by strong evidence (Table 1).

THE SUBJECTIVITY OF PHASE I OF CARDIAC REHABILITATION

When evaluating current protocols for phase I of cardiac rehabilitation, subjectivity regarding exercise prescriptions can be noted. The intensity is still determined in a subjective way[34], both for patients after cardiac surgery and for patients after acute myocardial infarction (AMI), using a quantitative index, which is the perceived effort index scale, or even the rating of fixed increments in the values for increase in heart rate, such as +30 bpm for post-acute myocardium infarction patients, or even +20 bpm for post-heart surgery patients[35,36]. In addition there are no specific descriptions of models for respiratory exercise prescriptions (essential for patients after surgery), where the series and repetitions are defined in a random way according to the patient's tolerance. In general the intervals between series are also defined by the patients, which is also subjective in relation to the activity and recovery time or according to the training density[36].

Respiratory exercises still fail to present standardized prescriptions of series repetitions, and a difficulty in adjusting the overload was noted, since the rating of the values for the increment in heart rate could signify different levels of exercise intensity. For example, 130 bpm for a 70-year-old individual with a resting HR of 65 bpm

Table 1 Presentation of end-points of papers disclosed since 1992 about evidence based on cardiopulmonary physiotherapy

Study	Design of study	Study objective	Study conclusions
Dean *et al*[28], 1992	Review study involving 61 articles	Examines advances in cardiopulmonary physiology and clinical medicine since the development of classic chest physical therapy practice, and the discordance of current physical therapy practices with the physiologic advances	(1) Cardiopulmonary physiology, pathophysiology and clinical medicine have advanced exponentially compared with physical therapy; (2) Establishing the efficacy of conventional chest physical therapy has been confounded by the lack of specificity of the underlying pathophysiology; (3) Needed to define the parameters for prescribing, position and mobilization so that the efficacy of these noninvasive interventions can be maximally explored in patient care
Stiller *et al*[29], 1994	Randomized controlled trial, involving 127 patients	Investigate whether prophylactic chest physical therapy affected the incidence of postoperative pulmonary complications	The results suggest that the necessity for prophylactic chest physiotherapy after routine coronary artery surgery should be reviewed
Johnson *et al*[30], 1996	Randomized controlled trial involving 78 patients	To determinate whether higher personnel intensive chest physical therapy can prevent the atelectasis that routinely follows cardiac valve surgery	The routine prescription of high intensity physical therapy does not improve patient outcomes but does add significantly to patient costs
Stiller[31], 2000	Review study involving 82 articles	To evaluate actuation of physiotherapy in intensive care	Although recommendations can be made concerning evidence based practice for physiotherapy, in the intensive care unit (ICU) these are limited because of the lack of data evaluating the effectiveness of physiotherapy in these settings. There is an urgent need for further research to be conducted to justify the role of physiotherapy in ICU
Wynne *et al*[32], 2004	Metanalyses involving 159 articles	To evaluate postoperative pulmonary dysfunction in adults after cardiac surgery	No single method of pulmonary physiotherapy is superior to others in preventing pulmonary complications
van der Peijl *et al*[33], 2004	Randomized controlled trial involving 246 patients	Compare the effectiveness of a low frequency program with high frequency and to assess whether the latter would yield sufficient benefit for the patient to justify higher costs in material end personnel	High frequency exercise program leads to earlier performance of functional tasks but would allow a sensible redistribution of the physiotherapists activity towards complicated and, therefore, more demanding patients
Pasquina *et al*[20], 2003	Review study involving 18 trials (1457 patients)	To assess whether respiratory physiotherapy prevents pulmonary complications after cardiac surgery	The usefulness of respiratory physiotherapy for the prevention of pulmonary complications after cardiac surgery remains unproved

represents an effort of 83.5% of the reserve heart rate (RHR) according to Karnoven's formula, whereas for a 55-year-old individual with the same resting HR of 65 bpm, it represents 75% of the RHR, according to the same formula. Similarly, an increment of 20 bpm during an exercise for an individual with a resting heart rate of 100 bpm can represent a different effort than the same increment for an individual with a resting HR of 80 bpm. This subjectivity in relation to the principles of overload, reversibility and specificity in exercise prescription, explicit in the protocols for phase I of cardiac rehabilitation, warrants a prospective study that would assess current models of exercise prescription. The scientific community should be aware of the lower level of evidence to assess the efficacy of physical therapy. Moreover, exercise prescription during the hospital phase has to be redefined.

The subjectivity in relation to the basics principles of exercise prescription in phase I of cardiac rehabilitation.

In 1768, Herbeden observed that a patient with chest angina significantly improved after wood chopping for half an hour every day. This finding, described by White[37], was one of the first publications referring to the benefits of physical activity on cardiopathies. Nevertheless physical activity as a form of treatment was put aside until the beginning of the twentieth century mainly because of the seriousness that cardiopathy represented to the patient's health. During the sixties, as bed rest was mandatory as part of the treatment in any cardiopathy[38,39] a few studies started demonstrating the deleterious effects of bed restriction not only physically, but also psychologically[40-42].

A trend for early mobilization took a while to become routine in the larger centers, and in 1969 a 3-wk bed rest period was still common after AMI. For years authors like Miller[43] argued that the myocardium continued to take risk when the muscle was precociously mobilized, since the removal of necrotic areas of the myocardium was not complete before the end of the fourth week and collateral circulation could take longer to be recruited. Such publications led many specialists to discuss the subject as the I International Congress on Cardiac Rehabilitation in Hamburg, in 1977, when the need for early mobilization was affirmed[44]. In 1993 the World Health Organization defined cardiac rehabilitation as the sum of the activities required to favorably influence both the subjacent cause of the disease and the physical, mental and social conditions of the patient, allowing patients to preserve or reassume their role in the community as soon as possible. As the years went by, cardiac rehabilitation programs grew in importance as a result of their[45-47], great social relevance[48,49]. According to Charlson *et al*[50], the increase of risk factors towards cardiovascular diseases among the elderly population increase the number of CABG procedures, which is associated with a significant reduction in

Table 2 Presentation of the ACSM recommendations for the prescription of exercises in phase I of cardiac rehabilitation

Intensity
TPE below 13 (scale 6-20)
Post AMI: HR below 120 bpm or resting HR + 20 bpm (Arbitrary lower limit)
Post-surgery: resting HR + 30 bpm (Arbitrary upper limit)
Up to tolerance if non-symptomatic
Duration
Intermittent sessions lasting from 3 to 5 min
Resting periods
As the patient wishes
Lasting from 1 to 2 min
Shorter than the time of the exercise sessions
Total duration of 20 min
Frequency
Early mobilization: 3 to 4 times per day (1st to 3rd days)
Subsequent mobilization: twice per day (As from the 4th day)
Progression
Initially increase the duration by up to 10 to 15 min of exercise time and then increase the intensity

AMI: Acute myocardial infarction; TPE: Table of perceived effort; HR: Heart rate; ACSM: American college of sports medicine.

physical capacity[48,49]. Seventy to 80% of patients submitted to heart surgery tend to recover their complete work capacity 1 year after procedure. This delay could be attributed to post-operative complications such as atelectasis, and pneumonia[51-53], which, amongst others, contributed to a reduction of physical aptitude of the patients during their hospital stay, resulting in a slower recovery[54-57]. According to American college of sports medicine (ACSM), cardiac rehabilitation can be divided into 4 phases[30].

Phase I, known as the hospital phase, aims to minimize the effects of restriction to bed and ends with hospital discharge. Phase II (up to 12 wk) starts immediately after discharge and is known as the early out-patient phase[47,48]. The aim is to develop activities that simulate the metabolic expense of everyday activities. Phase III, known as the late out-patient phase (variable duration) aims to develop exercises with more intensity[47,48]. The fourth and final phase is known as the preventive phase and should have a starting date but not a finishing one, where the patient will choose a cyclical activity of greater affinity, carrying out the program at least 3 times a week throughout one's lifetime[18].

Despite the fact that phase I is considered to be most important in the rehabilitation program, it presents a subjective prescription of exercises. Nevertheless it is one of the most used protocols throughout the world and is used as a study tool by many professionals. ACSM recommendations for the prescription of exercises in phase I of cardiac rehabilitation (both for patients after AMI and for patients after surgery) are shown in Table 2. The subjectivity of the phase I protocol can be perceived from the definition of the intensity of the exercises, where this should be controlled from the table of perceived effort (TPE). This table was developed by Borg (1979) with the objective of allowing the individual who exercises to subjectively classify the sensations during exercise, taking into account the level of personal aptitude, the environmental conditions and the level of fatigue[58,59]. There are currently two TPE scales in use, one classifying the exercise intensity from 6 to 20, and the revised one classifying it from 0 to 10. In addition to control by TPE, exercise intensity can be defined from the variation in heart rate, where post-AMI patients should increase their HR by a maximum of 20 and 30 bpm from resting during the exercise[60]. The control of the exercise intensity by the HR with a pre-determined target does not individualize the prescription of the exercise. Thus individualization, one of the principle fundamentals for the success of the exercise prescription[61], is not respected. In addition, the control of the perceived effort defined by the patient himself, may not picture the true energy consumption with respect to the activity. The value representing the effort can be super-estimated by the more apprehensive patients, or even sub-estimated by more motivated patients.

The intra-hospital protocol, in agreement with ACSM, suggests that an exercise session should last approximately 20 min. The session should be composed of intermittent series of exercises, each lasting between 3 and 5 min, with intervals between the series defined according to the wishes of the patient, or lasting between 1 and 2 min or even shorter than the duration of the exercise sessions. The interval between series appears as the main tool for the principle of reversibility or recovery, being as important as the definition of the series of repetitions. The interval should be defined from the volume and intensity of the exercise carried out, based on metabolic recovery of the energy sources and in the composition of the muscle fibers involved in movement[61]. Thus leaving the definition of the interval time to the discretion of the patient could, for example, generate unnecessary overload, resulting in prejudice to the patient. Thus, it's clear that the criteria for the definition of progression of the series and control of the intensity are still subjective[62]. The individualization of exercise prescription is essential, especially for patients after cardiac surgery. At this moment (phase I), patients have reduced cardiopulmonary reserve in regard to their exercise tolerance[63], meaning it is necessary to provide a judicious prescription controlling its specificity, overload and reversibility. These three basic principles of exercise prescriptions, as described by Bompa[64], must be respected so that the prescription can provide some benefit to the individual independent of his level of physical aptitude, be the patient a recently operated cardiac patient or an athlete.

The principle of specificity defines the effects of training. Improvement and potentiation of the predominant energy substrate system relies on its proper identification. Then an individualized exercise plan that meets the specific needs of the patient can be carried out. Thus the non-inclusion of a well-defined program of respiratory exercises for post-operative heart surgery patients represents the lack of specificity of the present method of phase I cardiac rehabilitation. As a result of median

sternotomy and the insertion of thoracic drainage tubes, these patients tend to present with a reduction of up to 30% in their pulmonary volumes and capacities. According to Guizilini *et al*[27], a decrease in forced ventilatory capacity can determine a fall in the expiratory flow peak, which also decreases immediately after surgery. This fall is clinically important since it will compromise the ability to cough and remove respiratory secretions[5]. The precocious closing and obstruction of the small airways predisposes the individual to microatelectasis and consequently to a reduction in the partial oxygen pressure in the arterial blood (PaO_2)[64,65]. Thus if pulmonary re-expansion is not rapidly reestablished, exercise tolerance will be promptly reduced[66-68]. The overload principle establishes that a tissue or organ has to be exposed to a load to which it is not used in order to improve its function (McKirnan *et al*[66]). Repeated exposition is associated with an adaptation on the part of the tissue or organ, which results in an improvement in the functional capacity. An exercise prescription should determine the intensity, duration and frequency of the training session meaning the interaction of these three variables determines the cumulative overload to which the tissue or organ should adapt[64].

INDIVIDUALIZATION OF EXERCISE PRESCRIPTION DURING HOSPITAL PHASE: A NEED TO BE ACHIEVED

The exercises should follow an adequate progression for organ or tissue adaptation. The activity can progress by progressive load model, where it is necessary to plan exercise sessions with the same characteristics in a determined time period or microcycle[64]. An increase in the load of exercises causes a slight physiological imbalance, followed by an adaptation phase, resulting in an improvement in performance[65]. The specificity of the prescribed exercises can directly influence the total session time, depending, obviously, on the clinical situation of the patient and on one´s level of physical aptitude[63]. One example is the prescription of respiratory exercises for post-operative heart surgery patients. In order to tolerate peripheral active exercises, many patients require specific respiratory work in order to potentially increase pulmonary volumes and capacities and gaseous exchange, consequently improving the distribution and capture of systemic oxygen and improving exercise tolerance[66].

Thus it is very difficult to standardize the session time without defining the specificity of the exercises or the work to be carried out with each patient[61]. The third basic principle of exercise prescription is that of reversibility[64]. This can be passive or active, where continuous low intensity exercises can help the metabolic recovery of the individual. In addition, the anatomical-physiological modifications that allow the individual to gradually increase his/her load intensity depend on the overload principle and on the pauses for recovery. The training programs should show intensity, duration and recovery periods that allow the organism to adapt so as to try and recover functional homeostasis (Barbanti[5]). Quite apart from the general principles for the adjustment of exercise prescriptions, the components of a session should also be respected. An exercise session should include a warming-up period, a specific work period referring to the initial objective of the work, a period of recreational activity and a winding-down period[65].

These components arise within an exercise session according to the objective of the prescribed series and the level of physical aptitude of the individual. The need to supervise the longitudinal evolution of an exercise prescription, respecting the general principles and aiming at constantly improving performance, motivated competitive sports trainers to develop a structure that helped in the organization and planning of the activities. Thus models for sports training periods were developed (Gomes[65]). The overload principle, directly responsible for the improvement in physical aptitude, can be represented by the increment in volume or in the intensity of the activity. A random increase in the exercise time, associated with a random definition of the interval between exercises, makes it difficult to prescribe the progression of the series. Thus the problem defining the overload activities for hospitalized individuals makes it difficult to program the series or divide it into periods, including the program of activities to be performed after discharge.

The methods for exercise prescription are represented by the strategies used to obtain a better yield or adaptation to the exercise. There are two main patterns: continuous and intermittent. Continuous exercise is of a submaximal nature and medium to long term, with intervals not permitted. The moderate intensity makes it possible to maintain the effort for a longer period of time. Intermittent exercise is characterized by a series of repeated periods of exercise alternated with periods of active rest or recovery intervals. The repeated series of exercises can have previously defined numbers, duration, intensity and intervals (McArdle *et al*[8]). It is important to point out that Kirkeby-Garstad *et al*[61] questioned the efficacy of low intensity activities in this phase of cardiac rehabilitation. The correct combination of stimulus intensity and duration with adequate recovery intervals allows the individual to support more intense activities.

PERIODIZATION IN HOSPITAL PHASE OF CARDIAC REHABILITATION: A NEW IDEA FOR PHYSICAL THERAPY BASED ON EVIDENCE

The difficulty found in demonstrating the results of a subjective program of prescribed exercises in the hospital phase (phase I), has contributed to the lack of adhesion to rehabilitation programs. Currently, less than 25% of the patients eligible for rehabilitation are enrolled in training programs in specialized centers in developed countries (Carlson *et al*[69]). After CABG these values

reach 25%-50% of the cases. When the number of patients taking part in supervised rehabilitation programs in developed countries is considered, 25%-50% give up after 6 mo and more than 90% after 1 year. The pertinent protocols have been shown to be non-executable in daily practice. Thus the need has arisen to redefine the methodology for prescribing exercises for phase I so as to motivate participants to continue in the rehabilitation program, and primarily to demonstrate objectivity in their prescription, respecting the basic exercise prescription principles.

Over the years, medicine has based itself on personal experiences, on the authority of more titled academics and on the descriptions of physiopathological theories. According to Atallah *et al*[70], evidence based medicine has arisen to guide decisions about health care. In a way, this conception of medicine removes the emphasis on practice based on intuition and on non-systematized clinical experience. Thus, according to Sackett *et al*[71], clinical reasoning in the form of research gains special distinction. In addition to new followers, evidence based physiotherapy is currently gaining importance since the activities of the professionals do not simply limit themselves to the clinical or medical diagnosis, but also include an evaluation of the repercussions or impact the disease brings to the life of the individual. Thus descriptive epidemiology becomes fundamental for the knowledge of a certain disease and its repercussions, where the physiotherapist should establish his/her diagnosis, prognosis, activity or treatment program, also carrying out interventions or reevaluations. The professional should fall back on books and periodicals to find the theoretical foundation to define the treatment program and apply a certain technique or exercise prescription methodology. The evidence levels produced in scientific studies are used as classification criteria according to the quality of studies in the health area[70].

Thus Wright *et al*[34] questioned the benefit of low intensity exercise for heart surgery patients. For his part, Hulzebos *et al*[72] stated that the physiotherapeutic techniques applied to post-heart surgery patients in the hospital phase, presented controversy regarding their efficacy in reducing the incidence of pulmonary complications. Britto *et al*[73] showed a significant lack of evidence regarding the effectiveness of physiotherapeutic techniques applied in the treatment of pulmonary empyema in the hospital phase, and, according to the author, the clinical decision taken by the physiotherapist was based on studies, the majority of which presented rudimentary methods, on personal experiences and on information obtained from books and from the opinions of professors and experts. Crowe *et al*[22] questioned the effectiveness of using inspiratory boosters associated with respiratory physiotherapy in the pulmonary re-expansion of patients with a high risk for respiratory complications, already in the hospital phase of patients who suffered an AMI and submitted to CABG[74], Overend *et al*[75] evaluated the effect of incentive spirometry (IS) on postoperative pulmonary complications (PPC), and it follows that the evidence does not support the use of IS for decreasing the incidence of PPC following cardiac or upper abdominal surgery. Thus it is apparent that some of the results referring to the prescription of exercises in cardio-respiratory physiotherapy are being questioned.

PHYSIOTHERAPIC APPROACH AND NEEDS

The physiotherapic approach and needs are different. The methods of prescribing exercises during in hospital phases need an urgent review, especially the methods related to cardiac surgery. Currently different needs such as individualization and periodization of exercise prescription during the hospital phase of cardiac rehabilitation should be studied, aiming to improve outcomes and levels of evidence regarding the application of techniques of cardiopulmonary physiotherapy. From the subjective way that is done today, exercise prescription at this stage has little effectiveness. It´s not possible to argue in the 21st century the efficacy of physical therapy in this field. Maybe the starting point for reviewing methods of prescription in phase I is to take a look at subjective quality related to the basic principle of exercise prescription: specificity, overload and reversibility. Importing the periodization applied on sports training programs and respecting the different levels of physical aptitude during the hospital phase may help improve the quality of prescription.

CONCLUSION

Thus phase I of cardiac rehabilitation has become a challenge for evidence based physiotherapy, having the means for success with the adjustment of a new exercise prescription model as long as this model is based on the principles of the clinical physiology of exercise, in the individualizing of the prescription. There is a real necessity for prospective randomized clinical trials in the physical therapy field. Maybe the way of programming the activities, creating a pattern including periods with well defined micro-cycles and series based on the application of functional tests, could guarantee greater objectivity, greater adherence to the programs and, as a consequence, better results.

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CASE REPORT

Apical hypertrophic cardiomyopathy

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Abstract

We describe a patient with asymptomatic apical hypertrophic cardiomyopathy (AHCM) who later developed cardiac arrhythmias, and briefly discuss the diagnostic modalities, differential diagnosis and treatment option for this condition. AHCM is a rare form of hypertrophic cardiomyopathy which classically involves the apex of the left ventricle. AHCM can be an incidental finding, or patients may present with chest pain, palpitations, dyspnea, syncope, atrial fibrillation, myocardial infarction, embolic events, ventricular fibrillation and congestive heart failure. AHCM is frequently sporadic, but autosomal dominant inheritance has been reported in few families. The most frequent and classic electrocardiogram findings are giant negative T-waves in the precordial leads which are found in the majority of the patients followed by left ventricular (LV) hypertrophy. A transthoracic echocardiogram is the initial diagnostic tool in the evaluation of ACHM and shows hypertrophy of the LV apex. AHCM may mimic other conditions such as LV apical cardiac tumors, LV apical thrombus, isolated ventricular non-compaction, endomyocardial fibrosis and coronary artery disease. Other modalities, including left ventriculography, multislice spiral computed tomography, and cardiac magnetic resonance imagings are also valuable tools and are frequently used to differentiate AHCH from other conditions. Medications used to treat symptomatic patients with AHCM include verapamil, beta-blockers and antiarrhythmic agents such as amiodarone and procainamide. An implantable cardioverter defibrillator is recommended for high risk patients.

Key words: Apical hypertrophic cardiomyopathy; Electrocardiogram

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INTRODUCTION

Apical hypertrophic cardiomyopathy (AHCM) is a rare form of hypertrophic cardiomyopathy (HCM) which usually involves the apex of the left ventricle and rarely involves the right ventricular apex or both[1]. Historically, this condition was thought to be confined to the Japanese population but it is also found in other populations. Of all the HCM patients in Japan the prevalence of AHCM was 15%, whereas in USA the prevalence was only 3%[2].

CASE REPORT

A 57-year-old white male with a history of hypertension, coronary artery disease, paroxysmal atrial fibrillation and metastatic squamous cell carcinoma of the tongue was

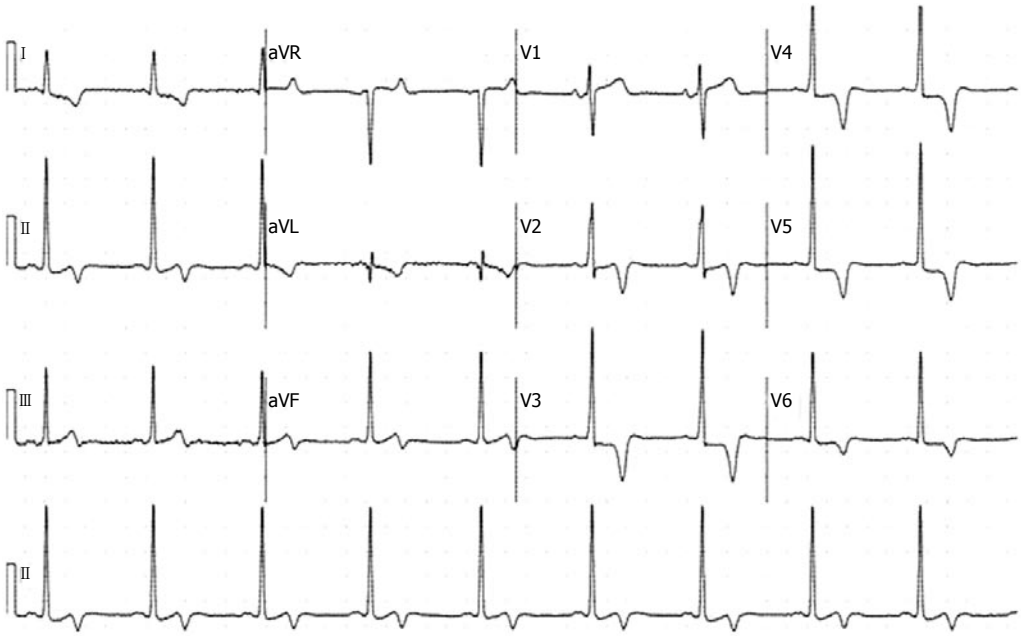

Figure 1 A 12-lead electrocardiogram showing left ventricular hypertrophy and inverted T-waves in the V2, V3, V4, V5 and V6 leads.

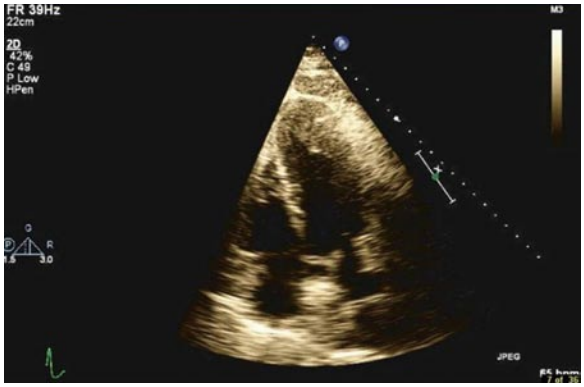

Figure 2 A 2D transthoracic echocardiogram showing left ventricular apical hypertrophy.

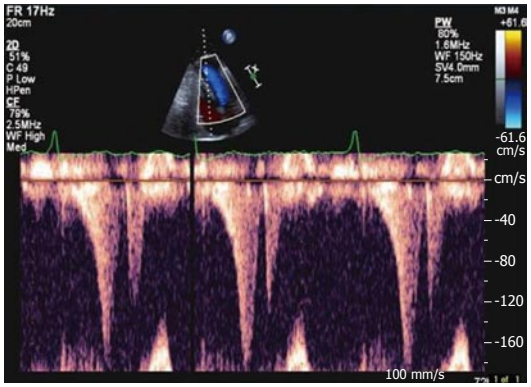

Figure 3 Doppler imaging showing a late peaking systolic gradient.

seen in March 2007 for preoperative evaluation. He had no complaints of chest pain, dyspnea or palpitations. There was no family history of sudden death, congestive heart failure or cardiomyopathy. On examination his blood pressure was 125/72 mmHg, heart rate 67 bpm with no heart murmur or any signs of congestive heart failure. The rest of the examination was normal. He was evaluated again in 2008 for altered mental status. His physical examination was unremarkable. A 12-lead electrocardiogram (ECG) showed left ventricular (LV) hypertrophy and inverted T-waves in V2 to V6 leads (Figure 1). All previous ECGs revealed similar findings. The cardiac enzymes and chest X-ray were normal. A transthoracic echocardiogram (TTE) showed apical hypertrophy with LV end diastolic diameter of 5.1 cm and normal systolic function (Figure 2). Doppler imaging showed a dagger-shaped late peaking systolic gradient, consistent with HCM (Figure 3). The ECG findings were attributed to AHCM. Since he was asymptomatic, no further investigations or particular treatment for this condition was carried out. One year later the patient developed episodes of atrial fibrillation and supraventricular tachycardia, for which he was treated with amiodarone. He had no other cardiovascular complications and was followed in the clinic, till he passed away in June 2009, due to widespread metastatic disease.

DISCUSSION

AHCM is frequently sporadic; however, a few families have been reported with autosomal dominant inheritance[3]. A sarcoma gene mutation (E101K mutation in the alpha-cardiac actin gene) has been identified in these families. A family history is more common in patients with asymmetric septal hypertrophy than with AHCM. Morphologically AHCM is divided into 3 types: pure focal, pure diffuse and mixed, of which pure focal is most common[4]. However in clinical practice this sub-classification is not widely accepted and its clinical relevance is

Table 1 Differential diagnosis of apical hypertrophic cardiomyopathy

Disease	Diagnostic tool to establish diagnosis of AHCM
Coronary artery disease	Echocardiogram/coronary angiogram and LVG
Left ventricular apical tumors	Echocardiogram with contrast/CCT/CMRI
Left ventricular apical thrombus	Echocardiogram with contrast/CCT/CMRI
Isolated ventricular non-compaction	CMRI/CCT
Endomyocardial fibrosis	LVG/CMRI

AHCM: Apical hypertrophic cardiomyopathy; CMRI: Cardiac magnetic resonance imaging; LVG: Left ventriculography; CCT: Cardiac computed tomography.

unknown. Others have divided AHCM into two groups, based on whether they had isolated asymmetric apical hypertrophy (pure AHCM) or had co-existent hypertrophy of the interventricular septum (mixed AHCM)[5]. The diagnostic criteria for AHCM included demonstration of asymmetric LV hypertrophy, confined predominantly to the LV apex, with an apical wall thickness ⩾ 15 mm and a ratio of maximal apical to posterior wall thickness ⩾ 1.5 mm, based on an echocardiogram or magnetic resonance imaging (MRI)[5].

The mean age of presentation of AHCM is 41.4 ± 14.5 years and is most commonly seen in males[5]. About 54% of patients with AHCM are symptomatic and the most common presenting symptom is chest pain, followed by palpitations, dyspnea and syncope[5]. AHCM may also manifest as morbid events such as atrial fibrillation, myocardial infarction, embolic events, ventricular fibrillation and congestive heart failure[5]. Other complications of AHCM include apical aneurysm and cardiac arrest. Physical findings of an audible/palpable fourth heart sound and a new murmur are common[5]. Our patient was asymptomatic, with no family history and had no physical findings.

The most frequent ECG findings are negative T-waves in the precordial leads which are found in 93% of patients, followed by LV hypertrophy in 65% of patients[5]. Negative T-waves with a depth > 10 mm are found in 47% of patients with AHCM[5]. The ECG in our patient showed LV hypertrophy and negative T-waves with a maximum depth of 7 mm. TTE shows hypertrophy of the LV apex and is the initial diagnostic tool for AHCM. When the baseline images are suboptimal, a contrast echocardiogram is useful in establishing the diagnosis[6]. On contrast ventriculography AHCM shows a distinctive LV "spade-like" configuration[5]. On single photon emission computed tomography (SPECT) myocardial perfusion imaging findings of resting "solar polar" map pattern and reduced flow reserve of the apex are the characteristics of AHCM[7]. Multislice spiral computed tomography can also be used to diagnose AHCM; besides diagnosis it provides information on cardiac anatomy, function and coronary arteries[8]. Cardiac MRI is also a valuable tool for diagnosing patients with inconclusive echocardiography and SPECT findings[9]. Although the initial diagnostic test for AHCM is most commonly TTE, the best diagnostic tool is considered to be cardiac MRI.

AHCM may mimic other conditions, including apical cardiac tumors[10], LV apical thrombus[11], isolated ventricular non-compaction[12], endomyocardial fibrosis (EMF)[13] and coronary artery disease[14] (Table 1). Chest pain in a patient with AHCM can be mistaken for ischemia from coronary artery disease[14]. Frequently these patients undergo a nuclear scan for abnormal ECG[15]. The majority of the patients with AHCM who suffer myocardial infarction have an apical infarct, and in these patients wall motion abnormalities varies from apical aneurysm to apical hypokinesis[5]. Some patients may have asymptomatic apical infarction[5]. Hence, in clinical practice, an apical aneurysm may sometimes be seen with AHCM in asymptomatic patients.

Isolated ventricular non-compaction may be differentiated from AHCM by high resolution images of the heart obtained by cardiac MRI[12]. An echocardiogram with contrast can be used to differentiate AHCM from a LV apical mass (thrombus or tumor)[11]. A LV angiogram shows apical obliteration during both systole and diastole in EMF, whereas in AHCM apical obliteration occurs only in systole and also there is an absence of significant ventricular hypertrophy in EMF patients[13].

In symptomatic patients with AHCM, verapamil, beta-blockers and antiarrhythmic agents are used[5]. Verapamil and beta-blockers are found to be beneficial in improving the symptoms in AHCM patients[16,17]. Amiodarone and procainamide are used in the treatment of atrial fibrillation and ventricular arrhythmias[18]. An implantable cardioverter defibrillator (ICD) is recommended for high risk HCM patients with (1) previous cardiac arrest or sustained episodes of ventricular tachycardia; (2) syncope; (3) a family history of sudden death; or (4) episodes of non-sustained ventricular tachycardia on serial Holter monitoring[19]. ICD has been was used in AHCM patients with cardiac arrest and non-sustained ventricular tachycardia[17].

Unlike other variants of HCM, the prognosis of AHCM is relatively benign. The overall mortality rate of AHCM patients was 10.5% and cardiovascular mortality was 1.9% after a follow-up of 13.6 ± 8.3 years[5]. Sudden death and cardiovascular events occur more commonly in patients with asymmetric septal hypertrophy than in those with AHCM[20]. A large LV end diastolic dimension may predict cardiac events in AHCM patients[21]. Some AHCM patients may develop sudden life-threatening complications, hence close follow-up of these patients is recommended[5].

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CASE REPORT

Coexistence of acute myocardial infarction with normal coronary arteries and migraine with aura in a female patient

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Abstract

Acute myocardial infarction with normal coronary arteries is a well known condition, which is typically diagnosed in young patients. Coronary vasospasm, inherited, acquired or malignancy-induced hypercoagulable state, collagen vascular disease and coronary arterial embolism have been considered as underlying etiologic factors. An association between migraine with aura and increased risk of ischemic stroke, angina and myocardial infarction has been demonstrated in studies. Patients with migraine and especially with aura should be followed closely against cardiovascular events even if they are young and do not have traditional risk factors.

Key words: Acute myocardial infarction; Aura; Migraine; Normal coronary arteries

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INTRODUCTION

Acute myocardial infarction (AMI) with normal coronary arteries is a well known condition, which has been described in the literature for many years. A number of hypotheses have been suggested to explain this condition. Coronary vasospasm, hypercoagulable state, collagen vascular disease and coronary arterial embolism have been considered as possible explanations. Migraine with aura (MWA) has been associated with an increased risk of ischemic stroke, angina and myocardial infarction. We report AMI with normal coronary arteries in a 50-year-old woman with a history of MWA who does not have traditional risk factors.

CASE REPORT

A 50-year-old woman was admitted to the hospital with complaints of retrosternal, squeezing chest pain lasting for 2 d. The patient had no known coronary artery risk factors. Her past medical history was significant for migraine headaches with nausea treated with a β-blocker for 5 years. Her electrocardiography (ECG) showed sinus rhythm without any ST-T wave abnormalities. Her blood chemistry was in the normal range except the troponin level, which was slightly elevated (0.65 ng/mL). Echocardiography showed normal left ventricular systolic function without any wall motion abnormality. Coronary angiography revealed normal coronary arteries (Figure 1A and B). The patient was discharged after 1 d with the diagnosis of Prinzmetal`s angina. The patient was subsequently admitted to hospital with complaints of aggravated chest pain

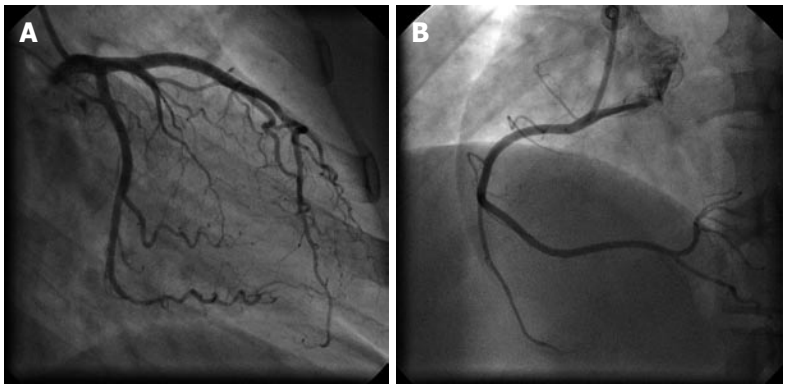

Figure 1 Coronary angiography. A: Normal left anterior descending artery and left circumflex artery; B: Normal right coronary artery.

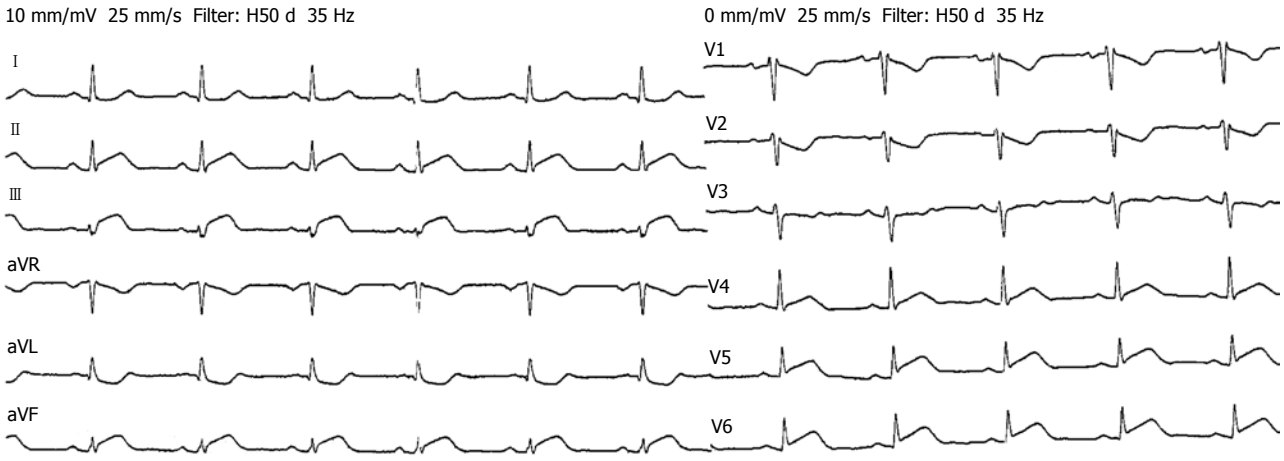

Figure 2 Electrocardiography showing acute inferolateral myocardial infarction.

compared to the first admission, accompanied by headache and nausea 20 h after the original discharge. ECG showed ST segment elevation in leads Ⅱ, Ⅲ, aVF and V4-6 with ST depression in leads Ⅰ, aVL and V1-2, which confirmed acute inferolateral myocardial infarction (Figure 2). There was no ST elevation in the right precordial leads. Thrombolytic therapy was not given as the coronary angiography 1 d before was normal and portable echocardiography revealed normal wall motion. Intravenous calcium channel blocker and nitrate treatment was started. 1 d after hospitalization troponin I level was 4.6 ng/mL. ECG revealed biphasic T waves in leads Ⅱ, Ⅲ and V4-6. Her control transthoracic echocardiography revealed mild hypokinesia in anterolateral and inferolateral walls with normal ejection fraction. The patient was discharged with calcium channel blocker and nitrate treatment.

DISCUSSION

AMI with normal coronary arteries is a well known condition, which is typically diagnosed in young patients. The prevalence of patients having AMI with normal coronary arteries has been reported as 2.8% in a recent study but there are differences in the prevalence between the published series[1,2]. This may relate to the various definitions of "normal" coronary arteries. Coronary vasospasm, inherited, acquired or malignancy-induced hypercoagulable state, collagen vascular disease and coronary arterial embolism were considered as the underlying etiologic factors[1,2]. However, an exact etiology could not be detected in a significant proportion of patients. There is no sex predilection and coronary artery risk factors are usually absent[1,3].

AMI with normal coronary arteries in young women who were under the hormonal influence of pregnancy or contraceptive pill usage has been reported in previous articles[4-6]. However, there was no pregnancy or history of contraceptive pill usage in our patient.

Migraine is a common neurologic disorder characterized by severe headache accompanied by autonomic dysfunction, nausea and vomiting. Transient neurologic symptoms before or during the headache attacks are known as migraine aura and they are most often visual[7]. An association between MWA and increased risk of ischemic stroke, angina and myocardial infarction has been demonstrated in a prospective cohort study. The age adjusted hazard ratios for ischemic stroke were 3.88 in the lowest risk score group and 1.00 for the highest. For myocardial infarction, these ratios were 1.29 and 3.34, respectively[7]. However the effects of migraine on the coronary arteries are still unknown.

Studies reported a high prevalence of patent foramen ovale (PFO) in patients with MWA[8,9]. Although PFO has been reported to be present in 40% to 60% of patients with MWA[10], there was no PFO in our patient.

A survey reported that migraine and coronary heart disease have a nonoverlapping age- and gender-specific prevalence[11]. Migraine is more prevalent in women than men throughout the lifespan and the peak prevalence of migraine occurs between ages of 30 and 49 years[11]. In a study, patients with migraine had lower blood pressure measured than those without migraine[12]. Antihypertensive drugs that are used in the treatment of migraine may control hypertension, which is a modifiable risk factor of coronary artery disease. The effects of antihypertensive drugs together with the nonoverlapping age- and gender-specific prevalence may result in a few patients with migraine experiencing myocardial ischemia.

Although the prognosis of patients with AMI and normal coronary arteries were reported as excellent in early reports, similar outcomes and prognosis in patients with normal coronary angiography and AMI compared with one- or two-vessel disease were reported in recent studies[1,13]. Also, similar mortality rates were reported in AMI patients with normal coronary arteries compared with patients with coronary artery stenosis[14].

Patients with migraine and especially with aura may have increased risk of myocardial infarction even with normal coronary arteries. They should be followed closely against cardiovascular events even if they are young and do not have traditional risk factors.

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Abdominal Obesity 2011 - 2nd International Congress on Abdominal Obesity
Buenos Aires, Argentina

Februrary 25-27
CardioRhythm 2011
Hong Kong, China

March 19-26
Cardiology Update: Caribbean Cruise
San Diego, CA, United States

March 25
Cardiology for General Practice
London, United Kingdom

April 1-2
11th Annual Spring Meeting on Cardiovascular Nursing
Brussels, Belgium

April 14-16
EuroPRevent 2011
Genova, Switzerland

April 30-May 4
ATC 2011 - 2011 American Transplant Congress
Philadelphia, United States

May 11-14
3th Radiochemotherapy and Brachitherapy Congress & 6th Medical Physycs Meeting
Córdoba, Argentina

May 15-18
ICNC10 - Nuclear Cardiology and Cardiac CT
Amstedan, The Netherlands

May 19-20
Adult Cardiovascular Pathology
London, United Kingdom

May 20-22
XXIX NATIONAL CARDIOLOGY CONGRESS
Córdoba, Argentina

May 20-22
4th Meeting Uremic Toxins and Cardiovascular Disease
Groningen, The Netherlands

May 21-24
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Gothenburg, Sweden

June 2-5
CODHy 2011 - The 1st Asia Pacific Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension
Shanghai, China

June 26-29
EHRA EUROPACE 2011
Madrid, Spain

June 29-July 1
Hands-on Cardiac Morphology - Summer Edition
London,
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August 27-31
ESC 2011 - European Society of Cardiology Congress 2011
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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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Books

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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