ISSN 2218-6204 (online)

World Journal of *Hematology*

World J Hematol 2016 November 6; 5(4): 75-98

Published by Baishideng Publishing Group Inc

World Journal of Hematology

A peer-reviewed, online, open-access journal of hematology

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2012-2016

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World Journal of Hematology

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NAME OF JOURNAL
World Journal of Hematology

ISSN
ISSN 2218-6204 (online)

LAUNCH DATE
June 6, 2012

FREQUENCY
Quarterly

EDITORIAL OFFICE
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World Journal of Hematology
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PUBLISHER
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Pleasanton, CA 94588, USA
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E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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PUBLICATION DATE
November 6, 2016

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World Journal of Hematology

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DOI: 10.5315/wjh.v5.i4.75

World J Hematol 2016 November 6; 5(4): 75-87
ISSN 2218-6204 (online)

ORIGINAL ARTICLE

Basic Study

Identifying changes in punitive transcriptional factor binding sites from regulatory single nucleotide polymorphisms that are significantly associated with disease or sickness

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Author contributions: Buroker NE analyzed all the data and wrote the manuscript.

Institutional review board statement: The manuscript did not use human or animal subjects.

Informed consent statement: The manuscript did not use human or animal subjects.

Conflict-of-interest statement: To the best of my knowledge, no conflict of interest exists.

Manuscript source: Invited manuscript

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Received: May 8, 2016
Peer-review started: May 9, 2016
First decision: June 13, 2016
Revised: June 24, 2016
Accepted: August 11, 2016
Article in press: August 13, 2016
Published online: November 6, 2016

Abstract

AIM

To identify punitive transcriptional factor binding sites (TFBS) from regulatory single nucleotide polymorphisms (rSNPs) that are significantly associated with disease.

METHODS

The genome-wide association studies have provided us with nearly 6500 disease or trait-predisposing SNPs where 93% are located within non-coding regions such as gene regulatory or intergenic areas of the genome. In the regulatory region of a gene, a SNP can change the DNA sequence of a transcriptional factor (TF) motif and in turn may affect the process of gene regulation. SNP changes that affect gene expression and impact gene regulatory sequences such as promoters, enhancers, and silencers are known as rSNPs. Computational tools can be used to identify unique punitive TFBS created by rSNPs that are associated with disease or sickness. Computational analysis was used to identify punitive TFBS generated by the alleles of these rSNPs.

RESULTS

rSNPs within nine genes that have been significantly associated with disease or sickness were used to illustrate the tremendous diversity of punitive unique TFBS that can be generated by their alleles. The genes studied are the adrenergic, beta, receptor kinase 1, the v-akt murine thymoma viral oncogene homolog 3, the activating transcription factor 3, the type 2 demodkinase gene, the endothetal Per-Arnt-Sim domain protein 1, the lysosomal acid lipase A, the signal Transducer and Activator of Transcription 4, the thromboxane A2 receptor and the vascular endothelial growth factor A. From this sampling of SNPs among the nine genes, there are 73 potential unique TFBS generated by the common alleles compared

to 124 generated by the minor alleles indicating the tremendous diversity of potential TFs that are capable of regulating these genes.

CONCLUSION

From the diversity of unique punitive binding sites for TFs, it was found that some TFs play a role in the disease or sickness being studied.

Key words: Regulatory single nucleotide polymorphisms; Alleles; Transcriptional factors; Transcriptional factor binding sites; Linkage disequilibrium; Disease or sickness

Core tip: Disease or trait-predisposing single nucleotide polymorphisms (SNPs) in or near genes can alter the transcriptional factor binding sites (TFBS) for the TFs regulating the gene; thereby affecting the health of an individual. In this report, the disease or sickness associated regulatory SNPs (rSNPs) within a sampling of nine human genes were studied with respect to the alterations in TFBS. From this sampling there were 73 punitive unique TFBS generated by the common rSNP alleles compared to 124 generated by the minor alleles indicating the tremendous diversity of potential TFs that are capable of affecting the health of person.

Buroker NE. Identifying changes in punitive transcriptional factor binding sites from regulatory single nucleotide polymorphisms that are significantly associated with disease or sickness. *World J Hematol* 2016; 5(4): 75-87 Available from: URL: http://www.wjgnet.com/2218-6204/full/v5/i4/75.htm DOI: http://dx.doi.org/10.5315/wjh.v5.i4.75

INTRODUCTION

The genome-wide association studies (GWAS) have over the past decade provided us with nearly 6500 disease or trait-predisposing single nucleotide polymorphisms (SNPs). Only seven percent of these SNPs are located in protein-coding regions of the genome[1,2] while the remaining 93% are located within non-coding regions[3,4] such as gene regulatory or intergenic areas of the genome. Much attention has been drawn to SNPs that occur in the putative regulatory region of a gene where a single nucleotide change in the DNA sequence of a potential transcriptional factor (TF) motif may affect the process of gene regulation[5-7]. A nucleotide change in a transcriptional factor binding site (TFBS) can have multiple consequences. Since a TF can usually recognize a number of different binding motifs in a gene, the SNP may not change the TFBS interaction with the TF and consequently not alter the process of gene expression. In other cases the nucleotide change may increase or decrease the TF's ability to bind DNA which would result in allele-specific gene expression. In some cases a nucleotide change may eliminate the natural binding motif or generate a new binding site (BS) as a result the gene is no longer regulated by the original TF[8,9]. Single nucleotide changes that affect gene expression by impacting gene regulatory sequences such as promoters, enhancers, and silencers are known as regulatory SNPs (rSNPs)[5,6,10,11]. Therefore, functional rSNPs in TFBS may result in differences in gene expression, phenotypes and susceptibility to environmental exposure[7]. Examples of rSNPs associated with disease susceptibility are numerous and several reviews have been published[7,12-16]. Advances in understanding the functional relevance of SNPs in non-coding regions of the human genome using epigenomics and genome engineering have been recently reviewed[17]. Computational tools can be used to identify punitive TFBS created by rSNPs which are associated with disease or sickness[18]. To this end, computational analysis has been used to identify punitive or potentially unique TFBS generated by the alleles of rSNPs[19] where unique TFBS occur with only one of the two rSNP alleles.

In this report, rSNPs within a sample of nine human genes (Table 1) which have been significantly associated with disease or sickness were selected to illustrate the tremendous diversity of unique punitive TFBS that can be generated by SNP alleles (Table 2)[8,9,20-27]. The SNP alleles from these reports were found to share common TFBS between alleles but each SNP allele can also create unique TFBS only for that allele (Table 2). As an example in Table 2, the rs948988 ADRBK1-G allele creates two potential unique TFBS for the Kruppel-like factors 1 and 4 (KLF1,4) TFs that do not occur with the alternate ADRBK1-A allele while the ADRBK1-A allele creates ten other punitive unique TFBS not found with ADRBK1-G allele. Many of the rSNPs have been reported to be in linkage disequilibrium (LD) (Table 1), where LD is considered to be the non-random association of SNP alleles within a gene. LD between SNPs in the regulatory region of a gene can indicate strong associations of certain haplotypes and TFBS with sickness or disease[28].

MATERIALS AND METHODS

Identifying TFBS

The JASPAR CORE database[29,30] and ConSite[31] were used to identify the TFBS in this study. JASPAR is a collection of transcription factor DNA-binding preferences used for scanning genomic sequences where ConSite is a web-based tool for finding cis-regulatory elements in genomic sequences. The Vector NTI Advance 11 computer program (Invitrogen, Life Technologies) was used to locate SNPs and TFBS within all genes listed in Table 1.

RESULTS

The protein and gene symbol, chromosome position of the gene, SNP number and location within the gene and nucleotide (mutation) change are listed in Table 1. Also listed is whether or not linkage disequilibrium occurs

Table 1 Genes and their single nucleotide polymorphisms that have been found to be associated with disease or sickness

Protein and gene symbol	Chromosome	SNP	SNP location	Mutation	LD	Ref.
Adrenergic, beta, receptor kinase 1	11q13.1	rs948988	intron 2	c.190 + 653G > A	Yes	[9,19]
		rs4370946	3'UTR	c.*217C > T	Yes	
v-akt murine thymoma viral oncogene homolog 3	1q44	rs4590656	intron 1	c.46 + 3654C > T	Yes	[8,19]
		rs10157763	intron 1	c.46 + 11386C > T	Yes	
		rs2125230	intron 1	c.47-26830G > A	Yes	
Activating transcription factor 3	1q32.3	rs3125289	promoter	c.-5 + 9322T > C	Unknown	[19,20]
		rs11119982	promoter	c.-4-23516C > T	Unknown	
Type 2 demodkinase gene	14q24.3	rs225015	3'UTR	c.*1453G > A	Yes	[19,21]
		rs225011	intron 1	c. 330 + 366C > T	Yes	
		rs12885300	5'UTR	c.-451C > T	Yes	
Endothetal Per-Arnt-Sim domain protein 1	2p21	rs6756667	intron 2	c.218-3881A > G	No	[22]
		rs1868092	3'UTR	c.*2403G > A	No	
Lysosomal acid lipase A	10q23.31	rs1412444	intron 2	c.229 + 2506C > T	n/a	[23]
Signal transducer and activator of transcription 4	2q32.3	rs8179673	intron 2	c.274-28290T > C	Yes	[24]
		rs10181656	intron 2	c.274-28828C > G	Yes	
Thromboxane A2 receptor	19p13.3	rs2238631	intron 1	c.-84 + 2229G > A	Yes	[19,25]
		rs2238632	intron 1	c.-84 + 2030C > T	Yes	
		rs2238634	intron 1	c.-84 + 1799G > T	Yes	
Vascular endothelial growth factor A	6p21.1	rs34357231	promoter	c. -2550-2568D > I	Yes	[19,26,28]
		rs1570360	promoter	c.-614A > G	Yes	
		rs3025039	3'UTR	c.*237C > T	Yes	

Also listed is the gene chromosome location, single nucleotide polymorphism location in the gene and the resulting genetic mutation as well as the occurrence of linkage disequilibrium between single nucleotide polymorphisms found within each gene. SNP: Single nucleotide polymorphism; LD: Linkage disequilibrium; n/a: Not available.

Table 2 Genes whose single nucleotide polymorphisms are significantly associated with human disease or sickness

Gene symbol							
ADRBK1	Ethnic group	B		B			
	disease or sickness	12		12			
	SNP	rs948988 (G/A)		rs4370946 (C/T)			
	alleles (MAF)	G	A (0.29)	C	T (0.2)		
	potential unique TFBS	KLF1, 4	BATF:JUN	E2F1,3,4,6	ARNT:AHR		
			ESR2	EGR1	ATOH1		
			FOS	INSM1	ELF1		
			FOSL2	KLF4	ESR2		
			JUND	NFKB1	NR3C1		
			JUN:FOS	NRF1			
			MYB	SP1, 2			
			NFE2L1:MAF				
			NR3C1				
			SOX17				
AKT3	Ethnic group	G		C		C	
	disease or sickness	1		14		14	
	SNP	rs4590656 (C/T)		rs10157763 (C/T)		rs2125230 (G/A)	
	alleles (MAF)	C	T (0.41)	C	T (0.33)	G	A (0.2)
	potential unique TFBS	ARNT:AHR	GFI	ELF5	CTCF	ARNT:AHR	GATA1
		HIF1a:ARNT	HNF4A	ELK1	NFATC2	FEV	HNF4a
			PAX2	MYCN	SOX17	HIF1a:ARNT	HOXA5
			SPIB	SPIB	ZNF354C	SPI1	IRF1
				SPI1			NR2F1
				TFAP2A			SOX17
ATF3	Ethnic group	C		C			
	disease or sickness	15		15			
	SNP	rs3125289 (C/T)		rs11119982 (C/T)			
	alleles (MAF)	C	T (0.10)	C	T (0.36)		
	potential unique TFBS	ARNT	FOXA1, 2	HLTF	ARID3A		
		ARNT:AHR	FOXL1		MAX		
		GABPa	FOXO3		MYB		
		MYC	HLTF		USF1		
		MYCN	SOX10		ZEB1		
		MZF1	SOX17				
		SPIB	SRY				
		USF1					

DIO2	Ethnic group	F		F		C	
	disease or sickness	2		2		17	
	SNP	rs225015 (G/A)		rs225011 (C/T)		rs12885300 (C/T)	
	alleles (MAF)	G	A (0.4)	C	T (0.42)	C	T (0.23)
	potential unique TFBS	EBF1	ELF1	CRX	FOXL1		ARID3A
		ESRRA	ELK1	RXRa	MEF2A		BATF:JUN
		PPARg:RXRa	ERG		PDX1		IRF1
		RFX5	ETS1				JUN:FOS
		THAP1	FLI1				PAX2
			RUNX1				SOX6
			SOX9				
			SPI1				
			TCF7L2				
EPAS1	Ethnic group	G		G			
	disease or sickness	1		1			
	SNP	rs6756667 (A/G)		rs1868093 (A/G)			
	alleles (MAF)	A	G (0.20)	A	G (0.25)		
	potential unique TFBS	CEBPa	ATF7	NR2C2	HIC2		
		NFIA	GMEB2	NFIA	KLF5		
		NRL	JDP2	YY1	MGA		
					TEAD1		
					USF1		
LIPA	Ethnic group	C, D, E					
	disease or sickness	20, 21					
	SNP	rs1412444 (C/T)					
	alleles (MAF)	C	T (0.32)				
	potential unique TFBS	ELF1	FOXA1				
		ETS1	FOXL1				
		GABPa	FOXO3				
		HOXA5	HNF1B				
		SPI1	MEF2A				
			NFKB1				
			NFIC				
			PAX2				
			SOX6				
			SOX9				
			SRY				
			THAP1				
STAT4	Ethnic group	A, C		A, C			
	disease or sickness	2,6,10		2,10			
	SNP	rs8179673 (T/C)		rs10181656 (C/G)			
	alleles (MAF)	T	C (0.26)	C	G (0.26)		
	potential unique TFBS	EN1	FOXA2	AR	HNF4g		
		NFIL3	FOXH1	E2F6	STAT3		
			FOXO1	NR1H3:RXRa			
			FOXP1	ZNF263			
			FOXQ1				
			HNF1a				
			HNF4g				
TBXA2R	Ethnic group	A		A		A	
	disease or sickness	22		22		22	
	SNP	rs2238631 (G/A)		rs2238632 (C/T)		rs2238634 (G/T)	
	alleles (MAF)	G	A (0.2)	C	T (0.21)	G	T (0.22)
	potential unique TFBS	FOXC1	ELK1		ARNT		HLTF
		TFAP2a	ELK4		CREB1		HNF4a
			ETS1		HIF1a:ARNT		NR2F1
			GATA2		MAX		NR2E3
			HAND1:		USF1		NR4A2
			TCFE2a				
			SPZ1				
VEGFA	Ethnic group	G		G		G	
	disease or sickness	1		1		1	
	SNP	rs34357231 (I/D)		rs1570360 (G/A)		rs3025039 (C/T)	
	alleles (MAF)	D	I (0.28)	G	A (0.13)	C	T (0.09)
	potential unique TFBS	HNF4a	AR	EGR1	EGR2	BRCA1	NFE2::MAF
		HNF4g	EGR1,2	MZF1	EHF	ESR2	RFX5
		JUN	KLF5	SP2	FOXH1	HIF1A:ARNT	YY1

		MYB	MZF1_1-4	MAFK	NFE2L1:MAFG
		NFIC	NFYB	SPIB	
		NR2C2	NFATC2	THAP1	
		NR4A2	NKX2-5		
		PAX2	NKX3-2		
		RFX5	SP1, 2		
			STAT5A:		
			STAT5B		
Ethnic Group	Disease		Disease		Disease
A. Asian	1. Chronic mountain sickness		8. Juvenile idiopathic arthritis		15. Hypospadias
B. Black	2. Diabetes		9. Primary biliary cirrhosis and Crohn's disease		16. Mental retardation
C. Caucasian	3. Hepatitis B virus- related hepatocellular		10. Lupus		17. Osteoarthritis
D. Chinese	4. Hepatitis B virus infection		11. Ulcerative colitis		18. Insulin resistance
E. Hispanic	5. HBV viral clearance		12. Cardiovascular disease		19. Hepatic glucose output
F. Pima Indians	6. Hepatocellular carcinoma		13. Renal cell carcinoma risk		20. Coronary artery disease
G. Tibetan	7. Inflammatory bowel disease		14. Aggressive prostate cancer		21. Myocardial infarction
					22. Asthma

Also listed are the SNP alleles and frequencies within the ethnic group as well as the potential unique transcriptional factor binding site created with each SNP allele. For a complete list of significant gene SNPs see references (Table 1). SNP: Single nucleotide polymorphisms; ADRBK1: Adrenergic, beta, receptor kinase 1; AKT3: V-akt murine thymoma viral oncogene homolog 3; ATF3: Activating transcription factor 3; DIO2: Type 2 demodkinase gene; EPAS1: Endothetal Per-Arnt-Sim domain protein 1; STAT4: Signal transducer and activator of transcription 4; TBXA2R: Thromboxane A2 receptor; VEGFA: Vascular endothelial growth factor A; LIPA: Lysosomal acid lipase A.

between the SNPs within each gene (Table 1). Nine genes, ethnic groups, disease or sickness, SNPs and alleles as well as potential unique TFBS per allele that have been reported are found in Table 2. Not all of the SNPs for each gene are listed in the tables but can be found in the accompanying references (Table 1). From Table 2, it can be seen that there occur incidences when the SNP common allele does not have any unique punitive TFBS while the minor allele provide several (*e.g.,* rs12885300 in DIO2; rs2238632 and rs2238634 in TBXA2R). There are other incidences where the SNP common allele provides one or two unique punitive TFBS while the minor alleles again provide several (*e.g.,* rs948988 in ADRBK1; rs4590656 in AKT3; rs11119982 in ATF3; rs8179673 in STAT4 and rs2238631 in TBXA2R). A near balance between SNP alleles in unique punitive TFBS can also be found in the table (*e.g.,* rs4370946 in ADRBK1; rs10157763 and rs2125230 in AKT3; rs3125289 in ATF3; rs6756667 in EPAS1; rs34357231 and rs3025039 in VEGFA). The minor allele of the SNP usually generates more punitive unique TFBS than the common allele (*e.g.,* rs948988 in ADRBK1; rs1412444 in LIPA and rs8179673 in STAT4). In fact, from this sampling of SNPs among the nine genes, there are 73 potential unique TFBS generated by the common alleles compared to 124 by the minor alleles (Table 2).

DISCUSSION

The possible relationship of these punitive unique TFBS to disease and sickness has previously been discussed for each gene in the accompanying references (Table 1). The use of rSNPs that are in LD within a gene to identify punitive TFBS can be illustrated with a few SNPs from these nine genes. The *ADRBK1* gene, which transcribes the GRK2 kinase, is an important regulator of beta-adrenergic signaling and plays a central role in heart failure (HF) pathology[32-34]. Two rSNPs in LD within the *ADRBK1* gene are rs948988 and rs4370946 whose minor alleles create punitive unique TFBS for ESR2 that is a binding site for the beta estrogen receptor which is expressed in blood monocytes and pulmonary epithelial cells (Tables 1-3). The ESR2 TFBS is not found with the common (rs948988 and rs4370946) alleles of the gene and may be related to HF. The NR3C1 TFBS for the glucocorticoid receptor which regulates carbohydrate, protein and fat metabolism is also only found with the minor alleles of these rSNPs (Tables 2 and 3) and should have an impact on HF. Other TFBS generated by the rs948988 minor allele of interest in HF might be the MYB and NFE2L.1:MAF TFs which are involved with hematopoietic progenitor cells and cell differentiation of erythrocytes as well as the rs4370946 common allele for the NRF1 TF which is involved with heme biosynthesis and mitochondrial DNA transcription and replication (Tables 2 and 3).

The type 2 demodkinase gene (*DIO2*) encodes a deiodinase that coverts the thyroid prohormone, thyroxine (T4), to the biologically active triiodothyronine (T3) where T3 plays an important role in the regulation of energy balance and glucose metabolism[35-38]. Two rSNPs in LD within the *DIO2* gene are rs225015 and rs225011 whose major alleles create unique punitive TFBS for TFs that are involved with energy balance and glucose metabolism (Tables 1-3). The ESRRα an alpha estrogen-related receptor that is involved with regulating thyroid hormone receptor genes while PPARα:RXRα and RXRα are involved with the regulation of adipocyte differentiation and glucose homeostasis (Tables 2 and 3). The minor allele of the rs225015 rSNP creates a unique punitive TFBS for the TCF7L2 TF whose protein is implicated in blood glucose homeostasis (Tables 2 and 3). The minor allele of the rs225011 rSNP creates a unique punitive TFBS for the PDX1 TF whose protein activates insulin, somatostatin, glucokinase, islet amyloid

Table 3 Transcriptional factors, protein name and their description or function

TF	Protein name	Transcriptional factor description/function
AR	Androgen receptor	The protein functions as a steroid-hormone activated transcription factor Upon binding the hormone ligand, the receptor dissociates from accessory proteins, translocates into the nucleus, dimerizes, and then stimulates transcription of androgen responsive genes. They are expressed in bone marrow, mammary gland, prostate, testicular and muscle tissues where they exist as dimers coupled to *Hsp90* and *HMGB* proteins
ARID3A	AT rich interactive domain 3A (BRIGHT-like)	This gene encodes a member of the AT-rich interaction domain family of DNA binding proteins
ARNT	Aryl hydrocarbon receptor nuclear translocator	Involved in the induction of several enzymes that participate in xenobiotic metabolism
ARNT:AHR	Hypoxia-inducible factor 1:Aryl hydrocarbon receptor nuclear translocator	The dimer alters transcription of target genes. Involved in the induction of several enzymes that participate in xenobiotic metabolism
ATF7	Activating Transcription Factor 7	Plays important functions in early cell signaling Has no intrinsic transcriptional activity, but activates transcription on formation of JUN or FOS heterodimers
ATOH1	Atonal homolog 1	Transcriptional regulator. Activates E box-dependent transcription in collaboration with TCF3/E47
BATF::JUN	Basic leucine zipper transcription factor, ATF-like Jun proto-oncogene	The protein encoded by this gene is a nuclear basic leucine zipper protein that belongs to the AP-1/ATF superfamily of transcription factors. The leucine zipper of this protein mediates dimerization with members of the Jun family of proteins. This protein is thought to be a negative regulator of AP-1/ATF transcriptional events
BRCA1	Breast cancer 1, early onset	This gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumor suppressor
CEBPA	CCAAT/enhancer binding protein, alpha	CCAAT/enhancer binding protein is a DNA-binding protein that recognizes two different motifs: the CCAAT homology common to many promoters and the enhanced core homology common to many enhancers
CREB1	cAMP responsive element binding protein 1	Phosphorylation-dependent transcription factor that stimulates transcription upon binding to the DNA cAMP response element, a sequence present in many viral and cellular promoters
CRX	Cone-rod homeobox	The protein encoded by this gene is a photoreceptor-specific transcription factor which plays a role in the differentiation of photoreceptor cells. This homeodomain protein is necessary for the maintenance of normal cone and rod function
CTCF	CCCTC-binding factor (zinc finger protein)	This gene is a member of the BORIS + CTCF gene family and encodes a transcriptional regulator protein with 11 highly conserved zinc finger domains. This nuclear protein is able to use different combinations of the zinc finger domains to bind different DNA target sequences and proteins
E2F1-6	E2F transcription factors 1-6	The protein encoded by this gene is a member of the E2F family of transcription factors. The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target of the transforming proteins of small DNA tumor viruses. The E2F proteins contain several evolutionally conserved domains found in most members of the family. These domains include a DNA binding domain, a dimerization domain which determines interaction with the differentiation regulated transcription factor proteins, a transactivation domain enriched in acidic amino acids, and a tumor suppressor protein association domain which is embedded within the transactivation domain
EBF1	Transcription factor COE1	EBF1 has been shown to interact with ZNF423 and CREB binding proteins
EGR1	Early growth response 1	The protein encoded by this gene belongs to the EGR family of C2H2-type zinc-finger proteins. It is a nuclear protein and functions as a transcriptional regulator. The products of target genes it activates are required for differentiation and mitogenesis
EGR2	Early growth response 2	The protein encoded by this gene is a transcription factor with three tandem C2H2-type zinc fingers Sequence-specific DNA-binding transcription factor
EHF	Ets homologous factor	This gene encodes a protein that belongs to an erythroblast transformation-specific transcription factor subfamily characterized by epithelial-specific expression. The encoded protein acts as a transcriptional repressor and may be involved in epithelial differentiation and carcinogenesis
ELF1	E74-like factor 1 (ets domain transcription factor)	The encoded protein is primarily expressed in lymphoid cells and acts as both an enhancer and a repressor to regulate transcription of various genes
ELF5	E74-like factor 5	A member of an epithelium-specific subclass of the Ets Transcription factor family
ELK1	ELK1, member of ETS oncogene family	This gene is a member of the Ets family of transcription factors and of the ternary complex factor subfamily. The protein encoded by this gene is a nuclear target for the ras-raf-MAPK signaling cascade
ELK4	ELK4, ETS-domain protein (SRF accessory protein 1)	This gene is a member of the Ets family of transcription factors and of the ternary complex factor subfamily. Proteins of the ternary complex factor subfamily form a ternary complex by binding to the serum response factor and the serum response element in the promoter of the c-fos proto-oncogene
EN1	Engrailed homeobox 1	Homeobox-containing genes are thought to have a role in controlling development

ERG	v-ets avian erythroblastosis virus E26 oncogene homolog	This gene encodes a member of the erythroblast transformation-specific family of transcriptions factors. All members of this family are key regulators of embryonic development, cell proliferation, differentiation, angiogenesis, inflammation, and apoptosis
ESR2	Estrogen receptor beta	Estrogen receptor β is a member of the family of estrogen receptors and the superfamily of nuclear receptor transcription factors and is expressed by many tissues including blood monocytes and tissue macrophages, colonic and pulmonary epithelial cells
ESRRA	Estrogen-related receptor alpha	This nuclear receptor acts as a site-specific transcription regulator and has been also shown to interact with estrogen and the transcripton factor TFIIB by direct protein-protein contact. The binding and regulatory activities of this protein have been demonstrated in the regulation of a variety of genes including lactoferrin, osteopontin, medium-chain acyl coenzyme A dehydrogenase and thyroid hormone receptor genes
ETS1	Protein C-ets-1	The protein encoded by this gene belongs to the erythroblast transformation-specific family of transcription factors and has been shown to interact with TTRAP, UBE2I and Death associated protein
FEV	ETS oncogene family	It functions as a transcriptional repressor
FLI1	Fli-1 proto-oncogene, ETS transcription factor	Sequence-specific transcriptional activator
FOS	FBJ murine osteosarcoma viral oncogene homolog	The Fos gene family consists of 4 members: FOS, FOSB, FOSL1, and FOSL2. These genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1. As such, the FOS proteins have been implicated as regulators of cell proliferation, differentiation, and transformation. In some cases, expression of the FOS gene has also been associated with apoptotic cell death
FOSL1 and 2	FOS-like antigen 1 and 2	GO annotations related to this gene include RNA polymerase II regulatory region sequence-specific DNA binding and sequence-specific DNA binding transcription factor activity
FOXA1	Forkhead box A1	Transcription factor that is involved in embryonic development, establishment of tissue-specific gene expression and regulation of gene expression in differentiated tissues. Is thought to act as a "pioneer" factor opening the compacted chromatin for other proteins through interactions with nucleosomal core histones and thereby replacing linker histones at target enhancer and/or promoter sites Involved in the development of multiple endoderm-derived organ systems such as liver, pancreas, lung and prostate. Modulates the transcriptional activity of nuclear hormone receptors
FOXA2	Forkhead box A2	Involved in embryonic development, establishment of tissue-specific gene expression and regulation of gene expression in differentiated tissues
FOXC1	Forkhead box C1	An important regulator of cell viability and resistance to oxidative stress in the eye
FOXL1	Forkhead box L1	Transcription factor required for proper proliferation and differentiation in the gastrointestinal epithelium. Target gene of the hedgehog signaling pathway
FOXO1	Forkhead Box O1	Transcription factor that is the main target of insulin signaling and regulates metabolic homeostasis in response to oxidative stress
FOXO3	Forkhead Box O3	This gene belongs to the forkhead family of transcription factors which are characterized by a distinct forkhead domain. This gene likely functions as a trigger for apoptosis through expression of genes necessary for cell death
FOXP1	Forkhead box P1	This gene belongs to subfamily P of the forkhead box transcription factor family. Forkhead box transcription factors play important roles in the regulation of tissue- and cell type-specific gene transcription during both development and adulthood. Transcriptional repressor. It plays an important role in the specification and differentiation of lung epithelium
FOXQ1	Forkhead box Q1	This gene belongs to the forkhead family of transcription factors which is characterized by a distinct DNA-binding forkhead domain. Plays a role in hair follicle differentiation
GABPA	GA-binding protein alpha chain	One of three GA-binding protein transcription factor subunits which functions as a DNA-binding subunit which shares identity with a subunit encoding the nuclear respiratory factor 2 gene and is likely involved in activation of cytochrome oxidase expression and nuclear control of mitochondrial function
GATA1	GATA binding protein 1	The protein plays an important role in erythroid development by regulating the switch of fetal hemoglobin to adult hemoglobin
GATA2	GATA binding protein 2	A member of the GATA family of zinc-finger transcription factors that are named for the consensus nucleotide sequence they bind in the promoter regions of target genes and play an essential role in regulating transcription of genes involved in the development and proliferation of hematopoietic and endocrine cell lineages
GATA3	GATA binding protein 3	Plays an important role in endothelial cell biology
GFI	Growth factor independent 1 transcription repressor	This gene encodes a nuclear zinc finger protein that functions as a transcriptional repressor. This protein plays a role in diverse developmental contexts, including hematopoiesis and oncogenesis. It functions as part of a complex along with other cofactors to control histone modifications that lead to silencing of the target gene promoters
GMEB2	Glucocorticoid modulatory element binding protein 1	This gene is a member of KDWK gene family. The product of this gene associates with GMEB1 protein, and the complex is essential for parvovirus DNA replication

HAND1: TCFE2α	Heart- and neural-crest derivatives-expressed protein 1: Transcription factor E2A	Hand1 belongs to the basic helix-loop-helix family of transcription factors The *Tcfe2a* gene encodes the transcription factor E2A, a member of the "class I" a family of basic helix-loop-helix transcription factors (also known simply as "E-proteins"). The transcription factor E2A controls the initiation of B lymphopoiesis
HIC1	Hypermethylated in cancer 1	This gene functions as a growth regulatory and tumor repressor gene
HIF1A:ARNT	Hypoxia-inducible factor 1: Aryl hydrocarbon receptor nuclear translocator	HIF1 is a homodimeric basic helix-loop-helix structure composed of HIF1a, the alpha subunit, and the aryl hydrocarbon receptor nuclear translocator (Arnt), the beta subunit. The protein encoded by HIF1 is a Per-Arnt-Sim transcription factor found in mammalian cells growing at low oxygen concentrations. It plays an essential role in cellular and systemic responses to hypoxia
HLTF	Helicase-like transcription factor	Member of the SWItch/Sucrose Non Fermentable family which have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering the chromatin
HNF1A	Hepatocyte nuclear factor 1 homeobox A	Transcriptional activator that regulates the tissue specific expression of multiple genes, especially in pancreatic islet cells and in liver
HNF1B	HNF1 homeobox B	This gene encodes a member of the homeodomain-containing superfamily of transcription factors. The protein binds to DNA as either a homodimer, or a heterodimer with the related protein hepatocyte nuclear factor 1-alpha. The gene has been shown to function in nephron development, and regulates development of the embryonic pancreas
HNF4α	Hepatocyte nuclear factor 4, alpha	The protein encoded by this gene is a nuclear transcription factor which binds DNA as a homodimer. The encoded protein controls the expression of several genes, including hepatocyte nuclear factor 1 alpha, a transcription factor which regulates the expression of several hepatic genes. This gene may play a role in development of the liver, kidney, and intestines
HNF4γ	Hepatocyte nuclear factor 4, gamma	Steroid hormone receptor activity and sequence-specific DNA binding transcription factor activity. An important paralog of this gene is RXRA
HOXA5	Homeobox protein Hox-A5	DNA-binding transcription factor which may regulate gene expression, morphogenesis, and differentiation
ISNM1	Insulinoma-associated 1	Insulinoma-associated 1 gene is intronless and encodes a protein containing both a zinc finger DNA-binding domain and a putative prohormone domain. This gene is a sensitive marker for neuroendocrine differentiation of human lung tumors
IRF1,2	Interferon regulatory factor	Members of the interferon regulatory transcription factor family that contain a conserved N-terminal region of about 120 amino acids, which folds into a structure that binds specifically to the interferon consensus sequence
JDP2	Jun dimerization protein 2	Component of the AP-1 transcription factor that represses transactivation mediated by the Jun family of proteins. Involved in a variety of transcriptional responses associated with AP-1 such as UV-induced apoptosis, cell differentiation, tumorigenesis and antitumogeneris
JUN	Jun Proto-Oncogene	This gene is the putative transforming gene of avian sarcoma virus 17. It encodes a protein which is highly similar to the viral protein, and which interacts directly with specific target DNA sequences to regulate gene expression
JUND	Jun D proto-oncogene	The protein encoded by this intronless gene is a member of the JUN family, and a functional component of the AP1 transcription factor complex. This protein has been proposed to protect cells from p53-dependent senescence and apoptosis
JUN::FOS	Jun proto-oncogene FBJ murine osteosarcoma viral oncogene homolog	Promotes activity of NR5A1 when phosphorylated by HIPK3 leading to increased steroidogenic gene expression upon cAMP signaling pathway stimulation Has a critical function in regulating the development of cells destined to form and maintain the skeleton. It is thought to have an important role in signal transduction, cell proliferation and differentiation
KLF1	Kruppel-like factor 1 (erythroid)	Transcription regulator of erythrocyte development that probably serves as a general switch factor during erythropoiesis. Is a dual regulator of fetal-to-adult globin switching
KLF4	Krueppel-like factor 4	Transcription factor that can act both as activator and as repressor. Regulates the expression of key transcription factors during embryonic development
KLF5	Krueppel-like factor 5	This gene encodes a member of the Kruppel-like factor subfamily of zinc finger proteins. The encoded protein is a transcriptional activator that binds directly to a specific recognition motif in the promoters of target genes. This protein acts downstream of multiple different signaling pathways and is regulated by post-translational modification. It may participate in both promoting and suppressing cell proliferation. Expression of this gene may be changed in a variety of different cancers and in cardiovascular disease. Alternative splicing results in multiple transcript variants
MAX	MYC associated factor X	The protein encoded by this gene is a member of the basic helix-loop-helix leucine zipper family of transcription factors

MAFK	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog K	Since they lack a putative transactivation domain, the small Mafs behave as transcriptional repressors when they dimerize among themselves. However, they seem to serve as transcriptional activators by dimerizing with other (usually larger) basic-zipper proteins and recruiting them to specific DNA-binding sites
MEF2A	Myocyte enhancer factor 2A	The protein encoded by this gene is a DNA-binding transcription factor that activates many muscle-specific, growth factor-induced, and stress-induced genes. Mediates cellular functions not only in skeletal and cardiac muscle development, but also in neuronal differentiation and survival
MGA	MGA, MAX Dimerization Protein	Functions as a dual-specificity transcription factor, regulating the expression of both MAX-network and T-box family target genes. Functions as a repressor or an activator
MYB	Myb proto-oncogene protein	This gene encodes a transcription factor that is a member of the MYB family of transcription factor genes. Transcriptional activator and plays an important role in the control of proliferation and differentiation of hematopoietic progenitor cells
MYC	v-myc myelocytomatosis viral oncogene homolog	The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation
MYCN	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	This gene is a member of the MYC family and encodes a protein with a basic helix-loop-helix domain. Amplification of this gene is associated with a variety of tumors, most notably neuroblastomas
MZF1_1-4	Myeloid zinc finger 1	Binds to target promoter DNA and functions as transcription regulator. May be one regulator of transcriptional events during hemopoietic development. Isoforms of this protein have been shown to exist at protein level
NFATC2	Nuclear factor of activated T-cells, cytoplasmic 2	This protein is present in the cytosol and only translocates to the nucleus upon T cell receptor stimulation, where it becomes a member of the nuclear factors of activated T cells transcription complex
NFIA	Nuclear Factor I/A	Recognizes and binds the palindromic sequence 5-TTGGCNNNNNGCCAA-3 present in viral and cellular promoters transcription and replication and in the origin of replication of adenovirus type 2. These proteins are individually capable of activating transcription and replication
NFIC	Nuclear factor 1 C-type	Recognizes and binds the palindromic sequence 5'-TTGGCNNNNNGCCAA-3' present in viral and cellular promoters and in the origin of replication of adenovirus type 2. These proteins are individually capable of activating transcription and replication
NFE2::MAF	Nuclear factor, erythroid 2 V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog	Regulates erythroid and megakaryocytic maturation and differentiation. Plays a role in all aspects of hemoglobin production from globin and heme synthesis to procurement of iron. When overexpressed, represses anti-oxidant response element-mediated transcription
NFE2L1: MAFG	Nuclear factor erythroid 2-related factor 1 Transcription factor MafG	Nuclear factor erythroid 2-related factor coordinates the up-regulation of cytoprotective genes via the antioxidant response element. MafG is a ubiquitously expressed small maf protein that is involved in cell differentiation of erythrocytes. It dimerizes with P45 NF-E2 protein and activates expression of a and b-globin
NFIL3	Nuclear factor, interleukin 3 regulated	Expression of interleukin-3 (MIM 147740) is restricted to activated T cells, natural killer cells, and mast cell lines
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	natural killer-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis
NFYB	Nuclear transcription factor Y, beta	The protein encoded by this gene is one subunit of a trimeric complex, forming a highly conserved transcription factor that binds with high specificity to CCAAT motifs in the promoter regions in a variety of genes. This gene product, subunit B, forms a tight dimer with the C subunit, a prerequisite for subunit A association. The resulting trimer binds to DNA with high specificity and affinity. Subunits B and C each contain a histone-like motif
NHLH1	Nescient helix loop helix 1	The helix-loop-helix proteins are a family of putative transcription factors, some of which have been shown to play an important role in growth and development of a wide variety of tissues and species
NKX2-5	Natural killer 3 homeobox 2	This gene encodes a member of the natural killer family of homeobox-containing proteins Transcriptional repressor that acts as a negative regulator of chondrocyte maturation
NKX3-2	Natural killer 3 homeobox 2	This gene encodes a member of the natural killer family of homeobox-containing proteins Transcriptional repressor that acts as a negative regulator of chondrocyte maturation
NR1H3:RXRa	Nuclear Receptor Subfamily 1, Group H, Member 3 Retinoid X receptor, alpha	The protein encoded by this gene belongs to the NR1 subfamily of the nuclear receptor superfamily The NR1 family members are key regulators of macrophage function, controlling transcriptional programs involved in lipid homeostasis and inflammation. This protein is highly expressed in visceral organs, including liver, kidney and intestine. It forms a heterodimer with retinoid X receptor, and regulates expression of target genes containing retinoid response elements Studies in mice lacking this gene suggest that it may play an important role in the regulation of cholesterol homeostasis

NR2C2	Nuclear receptor subfamily 2, group C, member 2	Orphan nuclear receptor that can act as a repressor or activator of transcription. An important repressor of nuclear receptor signaling pathways such as retinoic acid receptor, retinoid X, vitamin D3 receptor, thyroid hormone receptor and estrogen receptor pathways
NR2E3	Nuclear receptor subfamily 2, group E, member 3	This protein is part of a large family of nuclear receptor transcription factors involved in signaling pathways
NR2F1 (COUP)	Nuclear receptor subfamily 2, group F, member 1	Binds to the ovalbumin promoter and, in conjunction with another protein (S300-II) stimulates initiation of transcription. Binds to both direct repeats and palindromes of the 5'-AGGTCA-3' motif. An important paralog of this gene is RXRA
NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	Glucocorticoids regulate carbohydrate, protein and fat metabolism, modulate immune responses through suppression of chemokine and cytokine production and have critical roles in constitutive activity of the CNS, digestive, hematopoietic, renal and reproductive systems
NR4A2	Nuclear receptor subfamily 4, group A, member 2	Transcriptional regulator which is important for the differentiation and maintenance of meso-diencephalic dopaminergic neurons during development
NRF1	Nuclear respiratory factor 1	This gene encodes a protein that homodimerizes and functions as a transcription factor which activates the expression of some key metabolic genes regulating cellular growth and nuclear genes required for respiration, heme biosynthesis, and mitochondrial DNA transcription and replication
NRL	Neural Retina Leucine Zipper	This gene encodes a basic motif-leucine zipper transcription factor of the Maf subfamily. The encoded protein is conserved among vertebrates and is a critical intrinsic regulator of photoceptor development and function
PAX2	Paired box gene 2	Probable transcription factor that may have a role in kidney cell differentiation
PDX1	Pancreatic and duodenal homeobox 1	Activates insulin, somatostatin, glucokinase, islet amyloid polypeptide and glucose transporter type 2 gene transcription. Particularly involved in glucose-dependent regulation of insulin gene transcription
PPARγ:RXRα	Peroxisome proliferator-activated receptor gamma Retinoid X receptor, alpha	Peroxisome proliferator-activated receptor gamma is a member of the nuclear receptor family of ligand-activated transcription factors that heterodimerize with the retinoic X receptor to regulate gene expression. Peroxisome proliferator-activated receptor gamma is located primarily in the adipose tissue, lymphoid tissue, colon, liver and heart and is thought to regulate adipocyte differentiation and glucose homeostasis
RXRa	Retinoid X receptor, alpha	Retinoid X receptors and retinoic acid receptors, are nuclear receptors that mediate the biological effects of retinoids by their involvement in retinoic acid-mediated gene activation
RFX5	Regulatory factor X, 5	Activates transcription from class II MHC promoters. Recognizes X-boxes. Mediates cooperative binding between RFX and natural killer-Y. RFX binds the X1 box of MHC-II promoters
RUNX1	Runt-related transcription factor 1	Heterodimeric transcription factor that binds to the core element of many enhancers and promoters. The protein encoded by this gene represents the alpha subunit of core binding factor and is thought to be involved in the development of normal hematopoiesis
SOX6	SRY (sex determining region Y)-box 6	The encoded protein is a transcriptional activator that is required for normal development of the central nervous system, chondrogenesis and maintenance of cardiac and skeletal muscle cells
SOX9	SRY (sex determining region Y)-box 9	The protein encoded by this gene recognizes the sequence CCTTGAG along with other members of the involved in chondrogenesis by acting as a transcription factor for these genes
SOX10	SRY (sex determining region Y)-box 10	This gene encodes a member of the SRY-related HMG-box family of transcription factors involved in the regulation of embryonic development and in the determination of the cell fate
SOX17	SRY (sex determining region Y)-box 17	Acts as transcription regulator that binds target promoter DNA and bends the DNA
SP1	Specificity Protein 1	Can activate or repress transcription in response to physiological and pathological stimuli. Regulates the expression of a large number of genes involved in a variety of processes such as cell growth, apoptosis, differentiation and immune responses
SP2	Specificity Protein 2	This gene encodes a member of the Sp subfamily of Sp/XKLF transcription factors. Sp family proteins are sequence-specific DNA-binding proteins characterized by an amino-terminal trans-activation domain and three carboxy-terminal zinc finger motifs. This protein contains the least conserved DNA-binding domain within the Sp subfamily of proteins, and its DNA sequence specificity differs from the other Sp proteins. It localizes primarily within subnuclear foci associated with the nuclear matrix, and can activate or in some cases repress expression from different promoters
SPIB	Transcription factor Spi-B	SPI1 and SPIB are members of a subfamily of erythroblast transformation-specific transcription factors; erythroblast transformation-specific proteins share a conserved erythroblast transformation-specific domain that mediates specific DNA binding SPIB and SPI1 bind to a purine-rich sequence, the PU box (5-prime-GAGGAA-3-)
SPI1	Spleen focus forming virus proviral integration oncogene spi1	This gene encodes an erythroblast transformation-specific-domain transcription factor that activates gene expression during myeloid and B-lymphoid cell development
SPZ1		This gene encodes a basic helix-loop-helix-zip transcription factor which functions in the mitogen-activate protein kinase signaling pathway

SRY	Sex determining region Y	Transcriptional regulator that controls a genetic switch in male development
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	Signal transducer and transcription activator that mediates cellular responses to interleukins, KITLG/SCF and other growth factors
STAT5A: STAT5B	Signal transducer and activator of transcription 5A and transcription 5B	Carries out a dual function: signal transduction and activation of transcription Regulates the expression of milk proteins during lactation
TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)	This gene encodes a high mobility group box-containing transcription factor that plays a key role in the Wnt signaling pathway. The protein has been implicated in blood glucose homeostasis
TEAD1	TEA Domain Family Member 1	This gene encodes a ubiquitous transcriptional enhancer factor that is a member of the TEA/ATTS domain family. This protein directs the transactivation of a wide variety of genes and, in placental cells, also acts as a transcriptional repressor
TFAP2a	Activator protein 2	The AP2a protein acts as a sequence specific DNA-binding transcription factor recognizing and binding to the specific DNA sequence and recruiting transcription machinery
THAP1	THAP domain containing, apoptosis associated protein 1	DNA-binding transcription regulator that regulates endothelial cell proliferation and G1/S cell-cycle progression
USF1	Upstream transcription factor 1	This gene encodes a member of the basic helix-loop-helix leucine zipper family, and can function as a cellular transcription factor. The encoded protein can activate transcription through pyrimidine-rich initiator (Inr) elements and E-box motifs
YY1	YY1 transcription factor	YY1 is a ubiquitously distributed transcription factor belonging to the GLI-Kruppel class of zinc finger proteins. The protein is involved in repressing and activating a diverse number of promoters. YY1 may direct histone deacetylases and histone acetyltransferases to a promoter in order to activate or repress the promoter, thus implicating histone modification in the function of YY1
ZEB1	Zinc finger E-box-binding homeobox 1	A member of the delta-EF1 (TCF8)/Zfh1 family of 2-handed zinc finger/homeodomain proteins and interacts drosophila mothers against decapentaplegic proteins with receptor-mediated, activated full-length activated full-length drosophila mothers against decapentaplegic protein
ZNF263	Zinc finger protein 263	Might play an important role in basic cellular processes as a transcriptional repressor. An important paralog to ZNF496
ZNF354C	Zinc finger protein 354C	May function as a transcription repressor

polypeptide and glucose transporter type 2 gene transcription (Tables 2 and 3).

The thromboxane A2 receptor (*TBXA2R*) gene is a member of the seven-transmembrane G-protein-coupled receptor super family, which interacts with intracellular G proteins, regulates different downstream signaling cascades, and induces many cellular responses including the intracellular calcium influx, cell migration and proliferation as well as apoptosis[39]. Two rSNPs in LD within the *TBXA2R* gene are rs2238631 and rs2238634 whose minor alleles create unique punitive TFBS for TFs that are involved in signaling cascades and apoptosis (Tables 1-3). The ELK1 and SPZ1 TFs are involved with the ras-raf-MAPK signaling cascade while the ETS1 TF is involved with cell death (Tables 2 and 3). NR2E3 is part of a large family of nuclear receptor TFs involved in signaling pathways (Tables 2 and 3).

The other six genes can be analyzed in the same manner to identify punitive TFBS created by the rSNP alleles of these genes (Tables 2 and 3). What a change in the rSNP alleles can do, is to alter the DNA landscape around the SNP for potential TFs to attach and regulate a gene. This change in the DNA landscape can alter gene regulation which in turn can result in a change of a biological process or signaling pathway resulting in disease or illness. The process laid out in this report is a convenient way of identifying potential TFBS created by rSNP alleles that have been found to be significantly associated with disease or sickness. Any potential alterations in TFBS obtained by computational analyses need to be verified by protein/DNA electrophoretic mobility gel shift assays and gene expression studies[40]. CHIP-seq[41] experiments have become the standard method of validating TFBS and studying gene regulation[42-44].

In conclusion, SNPs in the regulatory region of a gene can alter the DNA landscape for TFs resulting in TFBS changes. Consequently, alterations in TF binding can affect gene regulation. Examples of this for nine genes are presented in this report where SNP alleles will either have no effect on TF binding or each allele will create unique punitive TFBS and alter a TFs ability to bind the DNA and regulate the gene.

COMMENTS

Background

Transcriptional factors (TFs) bind the DNA near a gene at transcriptional factor binding sites (TFBS) in order to regulate the gene. Single nucleotide polymorphisms (SNPs) that occur in the TFBS can alter the TFs ability to bind the DNA and thereby affect gene regulation. Such regulatory (r)SNPs have been associated with human disease and sickness. In this report, the alteration of TFBS created by rSNP alleles associated with disease has been documented for nine human genes. Sometimes the rSNP alleles will have no effect on the TFBS and not change the TF ability to bind the DNA. Other times each allele will create unique punitive TFBS that alter the TFs ability to regulate the gene.

Research frontiers

This article addresses an emerging concept in understanding how rSNPs which are significantly associated with disease can alter the TFBS for TFs that regulate a gene.

Innovations and breakthroughs

TFBS alteration by rSNPs is a newly emerging field of research and provides a different direction in examining changes in gene regulation resulting in human

disease and sickness.

Applications

Given the great diversity of punitive unique TFBS generated by each allele of a rSNP, the author suspects that alterations in TFBS affect how well a gene is expressed. The outcome may result in disease or sickness. The methods outlined in the article should be applied to all rSNPs that are associated with disease or sickness of a regulatory nature.

Terminology

rSNP: A regulatory single nucleotide polymorphism that affects gene expression; TF: Transcriptional factor that is involved with regulating a gene; TFBS: Transcription factor DNA binding site in the regulatory region of a gene; Unique TFBS: A TFBS created by one rSNP allele and not the alternate allele.

Peer-review

This study is technically well performed and a very interesting result. The interpretation was also sound. The report applied a computational approach to predict functional rSNPs in TFBS, focusing on several genes published earlier. Computational modeling and analysis for functional prediction is one of the approaches recently developed, particularly to address GWAS findings.

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P- Reviewer: Jeong BH, Xuei XL **S- Editor**: Qiu S **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Hematology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5315/wjh.v5.i4.88

World J Hematol 2016 November 6; 5(4): 88-93
ISSN 2218-6204 (online)

ORIGINAL ARTICLE

Basic Study

P2X7 receptor activation causes phosphatidylserine exposure in canine erythrocytes

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Author contributions: Kuit TA and Sluyter R designed the research; Faulks M, Sophocleous RA and Jurak LM performed the research; Faulks M, Sophocleous RA and Sluyter R analysed the data; Curtis BL and Curtis SJ contributed samples; Sluyter R wrote the paper; Faulks M, Sophocleous RA, Curtis BL, Curtis SJ and Jurak LM reviewed the paper.

Supported by The Centre for Medical and Molecular Bioscience (University of Wollongong); and the American Kennel Club Canine Health Foundation.

Institutional review board statement: The study was reviewed and approved by the Human and Animal Ethics Committees of the University of Wollongong.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Animal Ethics Committee of the University of Wollongong (Protocol AE14/09). Consent from pet owners was reviewed and approved by the Human Ethics Committee of the University of Wollongong (Protocol HE10/063).

Conflict-of-interest statement: The authors declare no conflicts of interest regarding this manuscript.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: July 20, 2016
Peer-review started: July 21, 2016
First decision: August 5, 2016
Revised: August 31, 2016
Accepted: September 21, 2016
Article in press: September 23, 2016
Published online: November 6, 2016

Abstract

AIM

To determine if activation of the ATP-gated P2X7 receptor channel induces phosphatidylserine (PS) exposure in erythrocytes from multiple dog breeds.

METHODS

Peripheral blood was collected from 25 dogs representing 13 pedigrees and seven crossbreeds. ATP-induced PS exposure on canine erythrocytes *in vitro* was assessed using a flow cytometric Annexin V binding assay.

RESULTS

ATP induced PS exposure in erythrocytes from all dogs

studied. ATP caused PS exposure in a concentration-dependent manner with an EC_{50} value of 395 μmol/L. The non-P2X7 agonists, ADP or AMP, did not cause PS exposure. The P2X7 antagonist, AZ10606120, but not the P2X1 antagonist, NF449, blocked ATP-induced PS exposure.

CONCLUSION
The results indicate that ATP induces PS exposure in erythrocytes from various dog breeds and that this process is mediated by P2X7 activation.

Key words: Adenosine triphosphate; Dog; P2X1 receptor; P2X7 receptor; Phospholipid; Purinergic receptor; Red blood cells

Core tip: Phosphatidylserine (PS) exposure in erythrocytes has potential roles in erythrocyte clearance and thrombus formation. Activation of the ATP-gated P2X7 receptor channel induces PS exposure in human erythrocytes, but whether this process occurs in erythrocytes from other mammals remained hitherto unknown. The current study shows that extracellular ATP causes PS exposure in dog erythrocytes from 13 pedigrees and seven crossbreeds. Notably, the current study shows that this process is mediated by P2X7 activation. These results suggest that P2X7-mediated PS exposure on erythrocytes may have important roles in red blood cell biology in dogs.

Faulks M, Kuit TA, Sophocleous RA, Curtis BL, Curtis SJ, Jurak LM, Sluyter R. P2X7 receptor activation causes phosphatidylserine exposure in canine erythrocytes. *World J Hematol* 2016; 5(4): 88-93 Available from: URL: http://www.wjgnet.com/2218-6204/full/v5/i4/88.htm DOI: http://dx.doi.org/10.5315/wjh.v5.i4.88

INTRODUCTION

Exposure of the plasma membrane lipid, phosphatidylserine (PS), to the outer leaflet is an important physiological and pathophysiological signal[1]. In erythrocytes, PS exposure serves emerging roles in the clearance of senescent, damaged and diseased erythrocytes from the circulation[2]. Moreover, PS exposure can serve as a substrate for thrombin formation and incorporation of erythrocytes into thrombi[3]. PS exposure also serves as a parameter for stored erythrocyte integrity[4] and may be important in the removal of such cells following transfusion[5]. Thus, it remains important to understand the mechanisms by which PS becomes exposed on the surface of erythrocytes.

The P2X7 receptor is a trimeric ligand-gated channel activated by extracellular ATP[6] at concentrations at least 10-fold greater than that required for other purinergic receptors[7]. Functional P2X7 has been reported in humans, dogs, rodents and other species[8]. P2X7 and other purinergic receptors, namely P2X1, P2Y1 and P2Y13, are present on the plasma membrane of erythrocytes[9]. P2X7 activation induces PS exposure in human erythrocytes[10,11], but it remains unknown if P2X7 activation mediates PS exposure in erythrocytes from other species. ATP can induce PS exposure in erythrocytes obtained from English springer spaniels[12], but whether this process occurs in other dog breeds and whether it is mediated by P2X7 activation remains to be determined. P2X7, however, is present in leukocytes from various dog breeds[13,14] suggesting that P2X7 activation may mediate PS exposure in canine erythrocytes.

Using a flow cytometric Annexin V binding assay, the current study aimed to determine if ATP induces PS exposure in erythrocytes from multiple dog breeds and whether this process is mediated by P2X7 activation.

MATERIALS AND METHODS

Materials

Nucleotides were from Sigma Chemical Co. (St. Louis, MO). AZ10606120 was from Tocris Bioscience (Ellisville, MO). NF499 was from Cayman Chemical (Ann Arbor, MI).

Blood samples

Peripheral blood was collected from either pedigree or crossbreed dogs into VACUETTE lithium heparin tubes (Greiner Bio-One, Frickenheisen, Germany). All samples were collected from privately owned dogs presenting at the Albion Park Veterinary Hospital (Albion Park, Australia), with informed consent of owners, and in accordance with and approval from the Animal and Human Ethics Committees of the University of Wollongong (Wollongong, Australia). The animal protocol was designed to minimize pain or discomfort to the animals, and conducted according to standard veterinary practices.

PS exposure assays

Erythrocytes from peripheral blood were isolated and resuspended in NaCl medium (147.5 mmol/L NaCl, 2.5 mmol/L KCl, 5 mmol/L glucose, 20 mmol/L HEPES, pH 7.4) at a final haematocrit of 2% as described[12]. Erythrocytes were then incubated in 96-well U-bottom plates (Greiner Bio-One) in the absence or presence of nucleotide (as indicated) for 24 h at 37 ℃/5% CO_2. In some experiments, erythrocytes were pre-incubated in the absence or presence of AZ10606120 or NF449 for 15 min at 37 ℃ prior to ATP addition. Following nucleotide incubation, 20 μL of resuspended erythrocytes were washed once in 1 mL Annexin V Binding Buffer (BioLegend, San Jose, CA) (450 × g for 3 min) and labeled with fluorescein isothiocyanate (FITC)-conjugated Annexin V (BioLegend, San Diego, CA) according to the manufacturer's instructions. Data was collected using a BD (San Jose, CA) LSR II or LSRFortessa flow cytometer

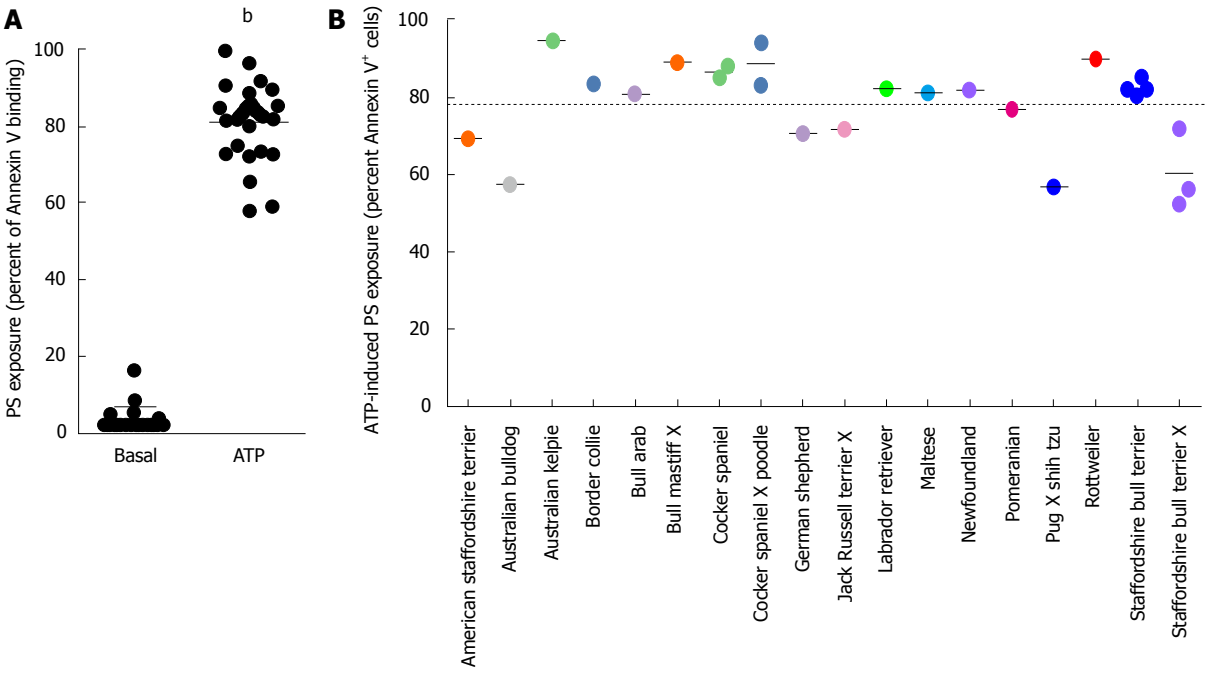

Figure 1 ATP induces phosphatidylserine exposure in erythrocytes from multiple dog breeds. Erythrocytes, from 17 pedigree and eight crossbreed (X) dogs, in NaCl medium were incubated for 24 h at 37 ℃ in the absence or presence of 1 mmol/L ATP. Cells were labeled with FITC-conjugated Annexin V and analyzed by flow cytometry. The bars represent group means (A and B). A: The symbols represent the percentage of Annexin V^+ erythrocytes, from each dog, following incubation in the absence (basal) or presence of ATP; $^bP < 0.0001$ ATP *vs* basal; B: The symbols represent the percentage of ATP-induced PS exposure in erythrocytes, from each dog, determined as the difference in the percentage of Annexin V^+ erythrocytes following incubation in the presence and absence of ATP. The broken line represents the mean ATP-induced PS exposure from all dogs. The symbols for Staffordshire bull terrier X represent a Staffordshire bull terrier and Australian kelpie cross, Staffordshire bull terrier and bull terrier cross, or a Staffordshire bull terrier cross. PS: Phosphatidylserine.

and FACSDiva software. The percentage of Annexin V^+ cells (PS exposure) was determined using FlowJo software (Tree Star, Inc., Ashland, OR).

Statistical analysis

Data is presented as mean ± SD. Statistical comparisons were performed using Prism 5 for Mac OS X (GraphPad Software, San Diego, CA). Differences between two or more groups were compared using a paired student's *t*-test or an ANOVA (using Tukey's multiple comparison test), respectively. Concentrations curves were fitted using the log(agonist) *vs* normalized response (variable slope) method.

RESULTS

To determine if ATP could induce PS exposure in erythrocytes in dog breeds other than English springer spaniels, erythrocytes, from 25 dogs representing 13 pedigrees and seven crossbreeds, were incubated in the absence or presence of ATP and the percent of Annexin V^+ cells (PS exposure) determined by flow cytometry. Incubation in the absence of ATP led to a mean PS exposure of 3.3% ± 3.2% (Figure 1A). In contrast, incubation with ATP caused a 25-fold increase in the mean PS exposure to 81.0% ± 10.4% (Figure 1A). Collectively, this resulted in an average ATP-induced PS exposure of 77.7% ± 11.8% (Figure 1B). Notably, ATP caused PS exposure in erythrocytes from all dogs studied (Figure 1B). ATP incubation also caused visible hemolysis compared to cells incubated in the absence of ATP (results not shown), but neither this nor other changes in erythrocyte morphology were investigated further.

To determine if P2X7 activation mediates exposure of PS in canine erythrocytes, erythrocytes were incubated with increasing concentrations of ATP and subsequent PS exposure assessed as described above. ATP induced PS exposure in a concentration-dependent manner with a maximum response at 2 mmol/L ATP and with an EC_{50} value of 395 ± 45 μmol/L (Figure 2A).

To further establish if P2X7 activation mediates PS exposure in canine erythrocytes, erythrocytes were incubated with ATP, as well as ADP and AMP, which do not activate canine P2X7[12,15]. Again ATP caused robust PS exposure in erythrocytes compared to erythrocytes incubated in the absence of nucleotide (Figure 2B). In contrast, ADP and AMP did not induce PS exposure in erythrocytes, with binding of Annexin V similar to that of erythrocytes incubated in the absence of nucleotide (Figure 2B).

Finally, canine erythrocytes were pre-incubated in the absence or presence of AZ10606120, which impairs canine P2X7[15], or NF449, which impairs human and rodent P2X1[16,17], prior to ATP incubation. Pre-incubation with AZ10606120 impaired ATP-induced PS exposure by 79%, while pre-incubation with NF449 had minimal effect on ATP-induced PS exposure (Figure 2C). Neither

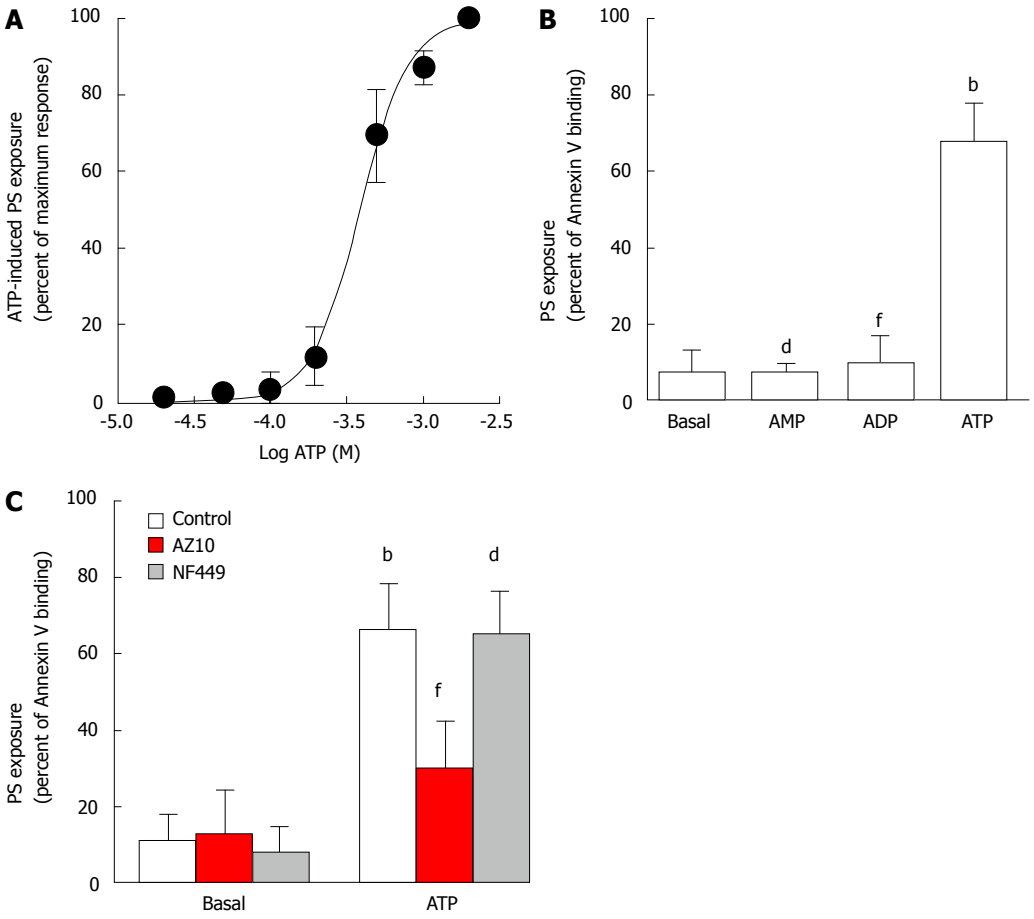

Figure 2 ATP induces phosphatidylserine exposure in canine erythrocytes in a concentration-dependent manner. Erythrocytes in NaCl medium were incubated for 24 h at 37 °C in the absence or presence of ATP (A) or 1 mmol/L nucleotide (B and C) (as indicated). Prior to ATP incubation, cells were pre-incubated for 15 min in the absence or presence of 10 μmol/L AZ10606120 (AZ10) or 10 μmol/L NF499 (C). Following nucleotide incubation, cells were labeled with FITC-conjugated Annexin V and analyzed by flow cytometry (A-C). A: The data represent percent maximum response to 2 mmol/L ATP (mean ± SD, *n* = 4 dogs); B: The data represent mean ± SD (*n* = 5 dogs); $^{b}P < 0.001$ ATP *vs* basal; $^{d}P < 0.001$ AMP *vs* ATP; $^{f}P < 0.001$ ADP *vs* ATP; C: The data represent mean ± SD (*n* = 5 dogs); $^{b}P < 0.001$ ATP *vs* basal; $^{d}P < 0.001$ ATP with NF449 *vs* NF449; $^{f}P < 0.001$ ATP with AZ10606120 *vs* ATP.

AZ10606120 nor NF449 affected PS exposure in the absence of ATP (Figure 2C).

DISCUSSION

The current study demonstrated that ATP induces PS exposure in erythrocytes from 25 dogs representing 13 pedigrees and seven crossbreeds. On average, ATP caused PS exposure on 78% of erythrocytes from these dogs. This value is similar to that of ATP-induced PS exposure previously observed in erythrocytes from English springer spaniels (88%)[12]. Combined, these data indicate that ATP can induce PS exposure in erythrocytes from multiple dog breeds and suggests that this is likely to be a common phenomenon in all breeds of dogs. Moreover, these data confirm that ATP-induced PS exposure in canine erythrocytes is about six-fold greater than that observed for ATP-induced PS exposure in human erythrocytes[12], which corresponds to the increased expression and activity of P2X7 in canine erythrocytes compared to human erythrocytes[12,18].

Similar to human erythrocytes[10,11], the current study also demonstrates that ATP-induced PS exposure in canine erythrocytes is predominately mediated by P2X7 activation. First, the EC_{50} value for ATP-induced PS exposure (395 μmol/L) is similar to that observed for native and recombinant canine P2X7-mediated cation fluxes in English springer spaniel erythrocytes[12,18] and transfected HEK-293 cells[15,19], respectively; second, the non-P2X7 agonists, ADP and AMP, did not cause PS exposure; last, the P2X7 antagonist, AZ10606120, but not the P2X1 antagonist, NF449, impaired ATP-induced PS exposure. It should be noted that blockade with AZ10606120 was not complete indicating that either other purinergic receptors have an additional role in this process, or that AZ10606120 has limited efficacy in the conditions tested and that P2X7 remains solely responsible for ATP-induced PS exposure in canine erythrocytes. The latter is supported by at least three points. First, the concentration response curve for ATP-induced PS exposure revealed a simple, not biphasic, sigmoidal curve suggesting involvement of only one purinergic receptor subtype. Second, ATP concentrations below 100 μmol/L, which are sufficient to activate other ATP-responsive purinergic receptors[7], failed to cause PS exposure. Last, ADP, which can activate P2X1, P2Y1 and

P2Y13, but not P2X7[7], all of which are present in human or rodent erythrocytes[20-22], did not induce PS exposure.

It remains unknown why the relative amounts of P2X7 differ between canine and human erythrocytes, but we have previously speculated[12] that this difference may be due to alterations in the proteolytic systems mediating maturation-associated degradation in reticulocytes between these two species. Differences in erythrocyte P2X7 activity between these two species are unlikely to be due to altered expression of splice variants. Previous immunoblotting studies using an antibody to the extracellular loop of P2X7, which is predicted to bind all known splice variants of canine P2X7 (URL: http://www.ncbi.nlm.nih.gov/gene/448778) and human P2X7[23,24], demonstrated only the full-length receptor in erythrocytes from both species[12]. Notably, the lifespans of canine and human erythrocytes are similar (approximately 115 d)[25] suggesting that P2X7-induced PS exposure in erythrocytes is unlikely to influence the removal of senescent cells.

In the current study, ATP caused visible hemolysis of canine erythrocytes, however this was not formally investigated. We have previously observed that 24 h ATP incubation induces a small but significant amount hemolysis of erythrocytes from English springer spaniels compared to those incubated in the absence of ATP (16% *vs* 1%, respectively)[12]. Future studies are required to explore if this ATP-induced hemolysis is mediated by P2X7 or other purinergic receptors, such as P2X1 or P2Y1, which can also mediate hemolysis[20,21]. Also, it remains unknown if 24 h ATP incubation causes other changes in erythrocyte morphology. Five minutes incubation with 1 mmol/L ATP of beagle erythrocytes increases cell viscosity as assessed by filterability of packed cells, but not changes in cell shape as observed by light microscopy[26]. Therefore, further studies could explore if activation of P2X7 or other purinergic receptors alters canine erythrocyte morphology.

In conclusion, the current study indicates that P2X7 activation induces PS exposure in canine erythrocytes and that this phenomenon is common to many, if not all, dog breeds. The physiological importance of P2X7-induced PS exposure in canine erythrocytes, as for human erythrocytes, remains to be established. The tendency of human erythrocytes to undergo ATP-induced PS exposure does not change with erythrocyte age[11], further supporting the concept that P2X7-induced PS exposure in erythrocytes is unlikely to be involved in the removal of aged cells. Instead, it remains plausible, that P2X7-induced PS exposure in erythrocytes is responsible for the clearance of these cells during cell stress, damage or disease. Alternatively, but not mutually exclusive to this point, P2X7-induced PS exposure in erythrocytes may facilitate thrombus formation to promote wound healing and immunity during tissue injury or infection, or to inadvertently cause vasocclusion in disorders such as malaria, sickle cell disease or diabetes. The robust PS exposure in canine erythrocytes following P2X7 activation will provide a valuable experimental model to understand further the role of this receptor in red blood cell biology. Finally, whilst PS exposure is routinely reported in canine platelets[27,28] and to some extent canine leukocytes[29,30], to the best of our knowledge PS exposure in canine erythrocytes is limited to our preliminary[12] and current observations. Thus, these studies support a rationale for exploring the physiological and pathophysiological roles and consequences of PS exposure in erythrocytes within dogs.

ACKNOWLEDGMENTS

The authors are grateful to Vanessa Sluyter (University of Wollongong) for technical assistance and pet owners for samples.

COMMENTS

Background

Exposure of phosphatidylserine (PS) in erythrocytes has roles in erythrocyte clearance and thrombus formation. Activation of P2X7 by extracellular adenosine triphosphate (ATP) induces PS exposure in human erythrocytes, but whether this process occurs in erythrocytes from dogs was unknown. Therefore this study aimed to determine if ATP can induce PS exposure in erythrocytes from dogs and if so, whether this process is mediated by activation of P2X7.

Research frontiers

The mechanisms by which PS exposure on dog erythrocytes and the function of P2X7 on these cells occurs remain poorly characterised. Moreover, there are limited reports of PS exposure on dog erythrocytes in any context.

Innovations and breakthroughs

This study demonstrated that extracellular ATP causes PS exposure in dog erythrocytes from multiple breeds and that this process is mediated by activation of P2X7.

Applications

This study suggests that P2X7-mediated PS exposure on erythrocytes may have important roles in red blood cell biology in dogs. This may have potential therapeutic or biomarker applications. Moreover, the relatively high amount of P2X7-mediated PS exposure on dog erythrocytes may provide a model to study this process, including its biological significance, in greater detail.

Terminology

PS is a phospholipid that is predominately localized to the inner layer of the lipid bilayer of the plasma membrane of healthy cells, but can become localized to the outer layer (exposed) following cellular activation. Annexin V is a PS-binding protein that can be conjugated to a fluorescent label and used to study cellular PS exposure by fluorescent techniques such as flow cytometry. The P2X7 receptor is a plasma membrane ligand-gated channel activated by extracellular ATP.

Peer-review

It is a well written interesting paper studying ATP-induced PS exposure, which has potential roles in erythrocyte clearance and thrombus formation, from various dog breeds and showing that this process is mediated by P2X7 activation.

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DOI: 10.5315/wjh.v5.i4.94

World J Hematol 2016 November 6; 5(4): 94-98
ISSN 2218-6204 (online)

CASE REPORT

Successful lower leg microsurgical reconstruction in homozygous sickle cell disease: Case report

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Author contributions: Posso C and Cuéllar-Ambrosi F contributed to the acquisition of data and writing of the manuscript.

Institutional review board statement: This case report was reviewed by and adhered to the standards set forth by the Institutional Review Board of the Clinic León XIII Ips University of Antioquia, Colombia.

Informed consent statement: The anonymity of the identity of the patient involved in this study was not compromised in the case report. The patient provided written informed consent for publication of these data.

Conflict-of-interest statement: Both authors declare no conflicts of interests in relation to this study or its publication.

Manuscript source: Unsolicited manuscript

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Received: May 21, 2016
Peer-review started: May 23, 2016
First decision: July 27, 2016
Revised: October 12, 2016
Accepted: October 25, 2016
Article in press: October 27, 2016
Published online: November 6, 2016

Abstract

We present an 18-year-old female with sickle cell disease, who presented with an extensive lower leg ulcer over a 12-year course of the disease. Definitive reconstruction was made using a free latissimus dorsi flap and split-skin grafts. One week before the surgery, the plasmapheresis protocol and blood transfusion were administered, in order to achieve a hemoglobin S level of $\leqslant$ 30%. Intraoperatively, the flap pedicle was rinsed with plasminogen activator inhibitor-1 until the thrombolytic agent was obtained from the comitant vein; after the arterial flow had been released, an intravenous bolus dose of heparin (2000 U) was administrated. No vascular complications occurred. Postoperatively, the patient received a 10-d course of hemodilution and a 14-d course of full-dose anticoagulation. After 8 mo postoperatively, the patient was able to walk and run, and showed complete wound healing. This case indicates that sickle cell disease is not a contraindication to free tissue transfer; however, the complications, their rate and overall outcomes for these cases are not yet clear. Herein, we provide an algorithm based on our clinical experience in this type of case and treatment, including several recommendations that may help to reduce thrombosis risk and systemic complications.

Key words: Sickle cell disease; Free flaps; Success rate; Microsurgical reconstruction; Ulcer

Core tip: This is a case report of a successful microsurgical

reconstruction in a patient with sickle cell disease who presented with an extensive lower leg ulcer during a 12-year course of the disease. We provide several recommendations for plasmapheresis and blood transfusions before the surgical reconstruction, and the anticoagulation protocol during the procedure and the postoperative period. This case description is intended to increase our colleagues' motivation to perform microsurgical reconstruction with a safer approach in the presence of hematologic diseases with elevated risk of thrombosis.

Posso C, Cuéllar-Ambrosi F. Successful lower leg microsurgical reconstruction in homozygous sickle cell disease: Case report. *World J Hematol* 2016; 5(4): 94-98 Available from: URL: http://www.wjgnet.com/2218-6204/full/v5/i4/94.htm DOI: http://dx.doi.org/10.5315/wjh.v5.i4.94

INTRODUCTION

The introduction of microsurgery has opened new fields for the reconstructive surgeon, so that we are now prepared to face even more challenging cases. During vascular anastomosis in microsurgical procedures, there is always risk of thrombosis, even in experienced hands and at prestigious institutions[1,2], but especially in cases of lower leg reconstruction. Patients with hematologic diseases and hypercoagulability are usually associated with high rates of complications, including anastomotic thrombosis and flap loss[3]. Clinical experience with free flaps and sickle cell disease is limited, and clear recommendations are not available.

Herein, we describe our clinical experience with a patient with sickle cell disease, who presented with a chronic ischemic lower leg ulcer that was reconstructed successfully with a free flap.

CASE REPORT

An 18-year-old Afro-American female from Chocó, Colombia, with known homozygous sickle cell disease consulted with our department regarding an extensive lower leg ulcer that had presented during a 12-year period. During that time, the patient had also presented with multiple episodes of limited osteomyelitis and soft tissue infections, for which she had been treated with systemic antibiotics; however, at no time had a real debridement and definitive reconstruction been performed because of the risk of complications (Figure 1).

One week before the microsurgical reconstruction, we started the patient on a plasmapheresis protocol and blood transfusion, with the aim of achieving a hemoglobin S level of ≤ 30%. An extensive soft tissue debridement and an anterior tibial decortication were performed subsequently, and no obvious bone defect was created. Bone and soft tissue cultures were taken; the analysis of which provided negative results. One

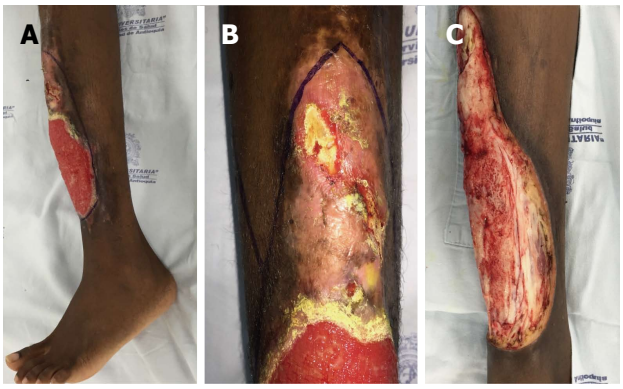

Figure 1 Ulcer on lower leg of the patient with sickle cell disease. A: Soft tissue defect; B: Detail of bone exposure; C: After extensive bone and soft tissue debridement.

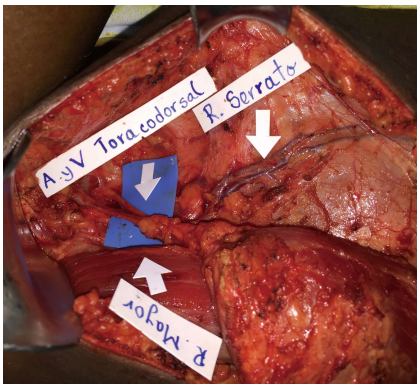

Figure 2 Vascular anatomy of the free flap.

week later, a free latissimus dorsi flap surgery was performed to cover the soft tissue defect (20 cm × 8 cm; Figure 2). The anterior tibial vessels were determined to be compromised at the middle-third of the lower leg. Before the anastomosis, the flap pedicle was rinsed using plasminogen activator inhibitor-1 (commonly known as PAI-1), until the thrombolytic agent was obtained from the comitant vein. An end-to-end anastomosis was then made between the posterior tibial artery and the thoracodorsal artery, and only one comitant vein was anastomosed. The venous anastomosis was made first following the arterial anastomosis, and later on an intravenous bolus dose of heparin (2000 U) was administered. The total ischemia time was 40 min. A partial skin graft was made to cover the muscle (Figure 3).

During the postoperative period, hemodilution was administered over a 10-d course; no vascular complications occurred. A 14-d course of full-dose anticoagulation (low-molecular-weight heparins) was administered as well. After 3 wk, the patient presented partial loss of the skin graft due to a superficial infection. The patient was admitted to the hospital for intravenous treatment with ciprofloxacin (400 mg/d). After 10 d, a second skin graft was made and complete healing was achieved. The final clinical result is shown in Figure 4,

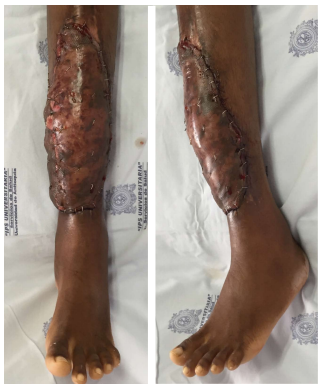

Figure 3 Immediate postoperative result. The muscle is shown from two angles covered with the split-thickness skin graft.

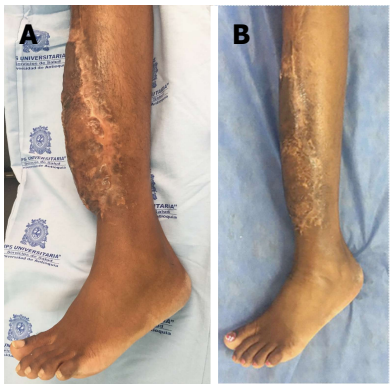

Figure 4 Follow-up postoperative results. A: After 3 mo of follow-up; B: After 8 mo of follow-up.

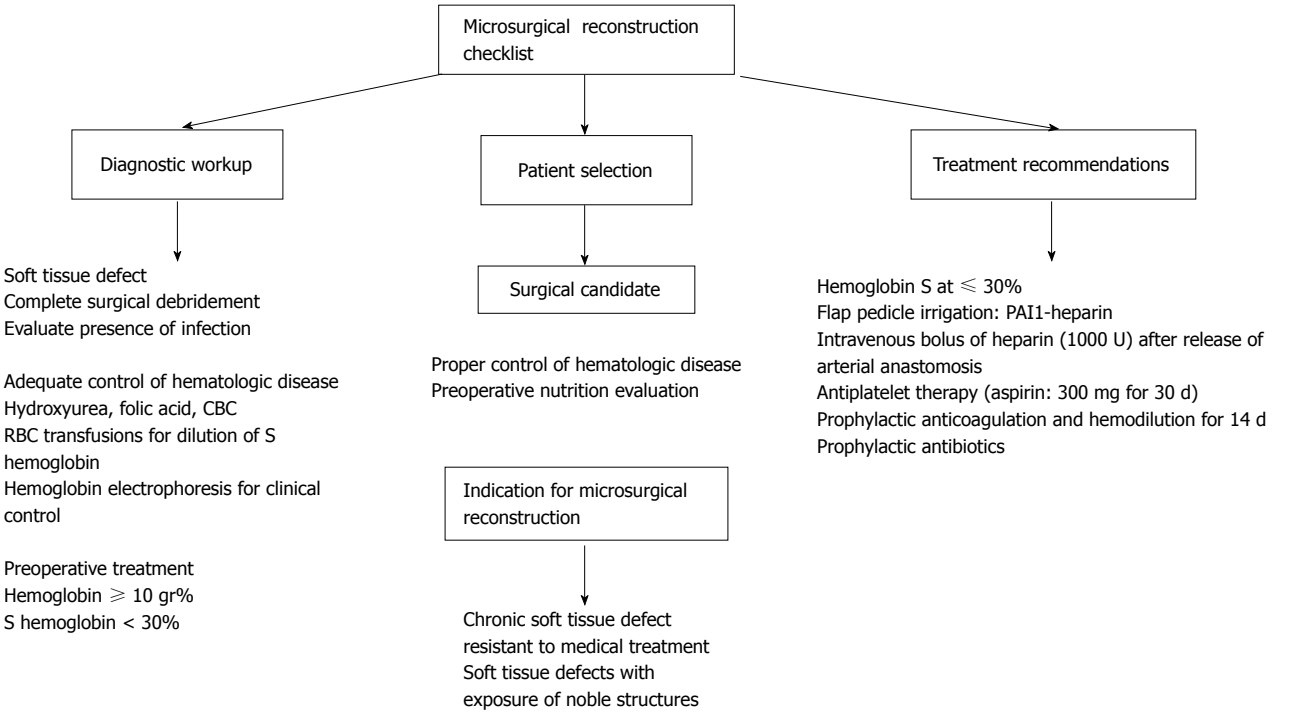

Figure 5 Algorithm treatment strategies for sickle cell disease.

after 3 mo and 8 mo of follow-up.

DISCUSSION

The sickle cell trait (SCT) results from the inheritance of one normal hemoglobin gene (HbA) and one mutated beta-globin gene (a sickle hemoglobin gene, HbS). In sickle cell disease, however, two sickle hemoglobin genes are inherited. The incidence of SCT and sickle cell disease in individuals living in the geographic region of Chocó, a Northwest Colombian area with a high density of Afro-American peoples, is 11%; intriguingly, vascular events are less frequent in these cases due to geographic adaptations of the sickle gene[4]. Increased erythrocyte sickling occurs under conditions of hypoxia, acidosis, dehydration and hypothermia[5]. SCT has been associated with an increased rate of exercise-related deaths, fetal loss, pre-eclampsia and venous thromboembolism.

Patients with SCT or sickle cell disease are usually considered a higher risk group for microsurgical reconstructions, and there is some pessimism among microsurgeons when these procedures are required[6]. In particular, rates of free flap loss in lower extremity reconstructions are higher compared to those in head and neck reconstruction, where the accepted rate of failure can be up to 5%. Failure rates reported in lower leg reconstruction, in contrast, varies from 6% to 15%, depending on the case series[7].

There is growing evidence from reports of clinical experiences involving patients with hematologic diseases and microsurgery[8], but most of the case series have thus far included patients with very different types of disorders (*i.e.,* various etiologies and risks

of thrombosis or bleeding), such as an increased number of blood elements responsible for hemostasis, abnormal blood elements or abnormality within the coagulation cascade. If we consider patients with sickle cell disease exclusively, only a few cases have been reported to date, some of which include flap loss[9,10]; hence, the question remains: Can we compare different hematologic diseases and outcomes in microsurgery?

According to our experience, there are several recommendations that should be included in this particular group of patients undergoing microsurgical reconstruction (Figure 5). In the first place, control of the medical condition is essential and the hematologist should work closely with the reconstructive surgeon. Normally, the patient receives multiple transfusions to maintain the HbS level well below the recommended 30% level for a free flap surgery so that the risk of anastomosis occlusion can be minimized[11]. On the other hand, it has been strongly recommended to use prophylactic anticoagulation therapy, but there is no evidence that this treatment will avoid any type of vascular complication. The ideal duration and type of therapy are not yet known.

Regarding the microsurgical technique, Ozkan *et al*[12] reported some strategies that would help to avoid complications, but we disagree on some of them. First, proper flap selection does not depend on whether or not your patient has any hematologic disease; reliable anatomy is always desirable, but donor site morbidity, extension and location of the soft tissue defect should be always considered. Ozkan *et al*[12] also recommended large recipient vessels, but what it is really important is to perform the vascular anastomosis in a healthy zone, avoiding inflammation of the vessel wall.

In conclusion, microsurgery transfer provides a well-vascularized tissue to solve extensive soft-tissue defects in a single procedure. In patients with SCT or sickle cell disease, complications such as thrombosis, bleeding, infection or poor wound healing may occur. This particular group of patients should be evaluated carefully before surgery so as to modify any potential risk factor. We have now demonstrated that it is possible to perform a successful free flap in one of these patients, but more extensive and closer monitoring in the postoperative period and a longer regimen of anticoagulation agents might be indicated. Moreover, this is a single-case report and larger case series are needed to clearly establish outcomes and rate of complications.

COMMENTS

Case characteristics

An 18-year-old female with medical history of sickle cell disease presented with a 12-year history of skin ulcer in her left leg and multiple episodes of limited osteomyelitis and soft tissue infections. The ulcer was reconstructed using a free flap.

Clinical diagnosis

A soft tissue defect was located over the anterior surface of the lower leg with bone exposure (20 cm × 8 cm).

Differential diagnosis

Chronic osteomyelitis, vascular ulcer.

Laboratory diagnosis

The hemoglobin level at admission was consistent with severe anemia.

Imaging diagnosis

A vascular occlusion was present in the anterior tibial artery.

Pathological diagnosis

After surgical debridement, bone culture was negative.

Treatment

Definitive soft tissue reconstruction was made using a free muscular flap and split-thickness skin graft.

Related reports

Microsurgical reconstruction in patients with hematologic diseases has not been reported frequently in the medical literature. Furthermore, if the authors consider patients with sickle cell disease exclusively, very few case reports are available, some of which include flap loss. There are no clear recommendations on how to decrease the risk of pedicle thrombosis in this patient population.

Term explanation

Sickle cell disease is an inherited red blood cell disorder, in which two sickle hemoglobin genes (HbS) are inherited. Increased erythrocyte sickling and secondary thrombosis occurs under special conditions, such as hypoxia, acidosis, dehydration and hypothermia, causing vascular compromise and skin necrosis in some cases. Patients with the sickle cell trait or disease are usually considered a higher risk group for microsurgical reconstructions, and preoperative control of the disease and a different surgical protocol should be included on order to avoid vascular complications.

Peer-review

This is an interesting and well written clinical case report.

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P- Reviewer: Carter WG, Siddiqui AH **S- Editor**: Kong JX
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