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SHORT COMMUNICATION

VEGFA rSNPs, transcriptional factor binding sites and human disease

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Abstract Three regulatory SNPs (rSNPs) in the promoter region of the vascular endothelial growth factor-A (*VEGFA*) gene have been significantly associated with several human diseases or conditions. The rSNP alleles alter the DNA landscape for potential transcriptional factors to attach, resulting in changes in transcriptional factor binding sites (TFBS). These TFBS changes are examined with respect to the human diseases which have been found to be significantly associated with the rSNPs.

Keywords *VEGFA* · rSNP · TFBS · Human disease

Introduction

Single nucleotide changes that affect gene expression by impacting gene regulatory sequences such as promoters, enhances, and silencers are known as regulatory SNPs (rSNPs) [1–4]. A rSNP within a transcriptional factor binding site (TFBS) can change a transcriptional factor's (TF's) ability to bind to its TFBS [5–8] in which case the TF would be unable to effectively regulate its target gene [9–13]. This concept is examined for three rSNPs (rs2010963, rs1570360 and rs699946) in the promoter region of the vascular endothelial growth factor (*VEGF*)-*A* gene and their allelic association with TFBS and human disease. The human *VEGFA* gene is encoded on chromosome 6 and is usually expressed as a 46-kDa disulfide-linked homodimer. VEGFA is a signaling protein involved in the regulation of angiogenesis, vasculogenesis and endothelial cell growth. It induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis, and induces permeabilization of blood vessels. The *VEGFA* rs2010963 [14–18], rs1570360 [15, 19–21] and rs699947 [18] rSNPs have been associated with several human diseases or conditions (Supplement). In this report, I discuss these rSNP associations with changes in potential TFBS and their possible relationship to the reported diseases or conditions. The potential TFBS for these rSNPs have previously been discussed in association with high altitude sickness [22].

Materials and methods

Identifying TFBS

The JASPAR CORE database [23, 24] and ConSite [25] were used to identify the TFBS in this study. JASPAR is a collection of transcription factor DNA-binding preferences used for scanning genomic sequences and ConSite is a web-based tool for finding cis-regulatory elements in genomic sequences. The TFBS and rSNP location within the binding sites have previously been discussed [22, 26]. The Vector NTI Advance 11 computer program (Invitrogen, Life Technologies) was used to locate the TFBS in the *VEGFA* gene (NCBI Ref Seq NM_001171626).

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Results

VEGFA rSNPs and TFBS

The rs2010963 rSNP (C/G) is located −634 base pairs (bp) from the *VEGFA* TSS, while the rs1570360 rSNP (A/G) is at −1,154 bp and the rs699947 rSNP (A/C) is at −2,578 bp (Table 1). The rSNPs are located in several TFBS which have previously been reported [22]. Sometimes the rSNP alleles do not change the TFBS but in other instances each allele may provide a unique TFBS such as shown in Table 1. As an example, the rs2010963 *VEGFA*-C allele generates the potential binding sites for the GA-binding protein alpha (GAPBα) and the interferon regulatory factors 1 and 2 (IRF1, 2) TFs, while the *VEGFA*-G allele generates a potential binding site for the specificity protein 1 (SP1) TF. The rs1570360 *VEGFA*-A allele generates the potential bindings sites for the SP1 and zinc finger protein 354C (ZNF354C) TFs, while the *VEGFA*-G allele generates potential binding sites for Krueppel-like factor 4 (KLF4) and methyl-CpG-binding protein 2-interacting zinc finger protein (MIZF) TFs. The rs699947 *VEGFA*-A allele generates a potential binding site for the nuclear factor 1 C-type (NFIC) TF while the *VEGFA*-C allele generates potential binding sites for GATA binding protein 3 (GATA3), the hypoxia-inducible factor 1::aryl hydrocarbon receptor nuclear translocator (HIF1α::ARNT) and the T cell acute lymphocytic leukemia 1::transcriptional factor 3 (TAL1::TCF3) TFs. The function of these TFs has previously been reported and discussed [22].

VEGFA rSNP alleles and disease associations

A number of human diseases or conditions have significantly been associated with *VEGFA* rSNP alleles shown in bold lettering in the table along with the potential TFBS generated by the specific allele. These diseases or conditions have been listed along with the *VEGFA* rSNP

Table 1 Human diseases and VEGFA rSNPs found to be significantly associated in the referenced study

Disease/condition	rSNP distance from TSS	rSNP	Allele	TFBS	Reference
Severe ischemic complications in GCA	(−) 634 bp	rs2010963	C	GABPa, IRF1,2	[14, 27]
			G	SP1	
Henoch–Schonlein purpura + nephritis			**C**		[15]
			G		
Ventricular septal defect			C		[16]
			G		
Recurrent depressive disorder			**C**		[17]
			G		
Coronary artery disease			**C**		[18]
			G		
Henoch–Schonlein purpura + nephritis	(−) 1,154 bp	rs1570360	A	SP1, ZNG354C	[15]
			G	KLF4, MIZF	
Henoch–Schonlein purpura + renal sequelae			A		[15]
			G		
Proliferative diabetic retinopathy			**A**		[19]
			G		
Sporadic Alzheimer's disease			A		[20]
			G		
Hypertensive nephropathy			**A**		[21]
			G		
Coronary artery disease	(−) 2,578 bp	rs699947	**A**	NFIC	[18]
			C	GATA3, HIF1a:ARNT, TAL1:TCF3	

rSNP alleles alter the transcriptional factor binding sites (TFBS) in non-coding regulatory regions of the gene. The rSNP alleles are found only in these TFBS. Alleles in bold are increased in patients compared to controls. TSS is the *VEGFA* transcriptional start site

genotypes and allele frequencies for patients with the disease versus their controls (Supplement). The allele listed in bold lettering in the table is shown to increase in disease patients compared to the controls (Supplement). As an example, the rs2010963 *VEGFA*-G allele has been found to significantly increase in patients with severe ischemic complications in giant cell arteritis (GCA) [27]. This allele generates the potential SP1 binding site (Table 1), where SP1 can activate or repress transcription in response to physiological and pathological stimuli. SP1 can regulate the expression of a large number of genes involved in a variety of processes such as cell growth, apoptosis, differentiation and immune responses, whereas the rs2010963 *VEGFA*-C allele has been found to significantly increase in patients with coronary artery disease (CAD) [18]. This allele generates the potential GAPBα and the IRF1, 2 binding sites (Table 1), were GAPBα is involved in activation of cytochrome oxidase expression and has nuclear control of mitochondrial function, and IRF1, 2 are involved in interferon regulation. The rs1570360 *VEGFA*-A allele has been found to significantly increase in patients with proliferative diabetic retinopathy (PDR) compared to their controls [19]. This allele generates the potential SP1 and ZNF354C binding sites, where the ZNF354C TF functions as a transcriptional repressor. The rs1570360 *VEGFA*-G allele has been found to significantly increase in patients with sporadic Alzheimer's disease (SAD) [20]. This allele generates the potential KLF4 and MIZF binding sites, where KLF4 acts as both an activator and repressor and MIZF plays a role in DNA methylation and transcription repression. The rs699947 *VEGFA*-A allele has been found to significantly increase in CAD patients compared to their controls [18]. This allele generates the potential NFIC binding site. NFIC is a member of the NFI gene family and is expressed in numerous tissues including brain, liver, spleen and heart [28]. The proteins from these genes are individually capable of activating transcription and replication. Other significant disease associations with these *VEGFA* rSNP alleles are listed in Table 1.

Discussion

GWAS over the last decade have identified nearly 6,500 disease or trait-predisposing SNPs where only 7 % of these are located in protein-coding regions of the genome [29, 30] and the remaining 93 % are located within non-coding areas [31, 32], such as regulatory or intergenic regions. SNPs which occur in the putative regulatory region of a gene where a single base change in the DNA sequence of a potential TFBS may affect the process of gene expression, are drawing more attention [1, 3, 33]. A SNP in a TFBS can have multiple consequences. Often, the SNP does not change the TFBS interaction nor does it alter gene expression, since a TF will usually recognize a number of different binding sites in the gene. In some cases the SNP may increase or decrease the TF binding which results in allele-specific gene expression. In rare cases, a SNP may eliminate the natural binding site or generate a new binding site, in which cases the gene is no longer regulated by the original TF. Therefore, functional rSNPs in TFBS may result in differences in gene expression, phenotypes and susceptibility to environmental exposure [33]. Examples of rSNPs associated with disease susceptibility are numerous and several reviews have been published [33–36].

Human diseases or conditions that have been significantly associated with rSNPs of the *VEGFA* gene are shown in Table 1, along with rSNP allele-specific TFBS. 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 regulatory landscape can alter gene regulation which in turn can result in human disease, a change in condition or illness. In this report several examples have been described to illustrate that a change in rSNP alleles can provide different TFBS, which in turn are also significantly associated with human disease.

Conflict of interest The author has no conflicts of interest or financial ties to disclose.

References

1. Knight JC (2003) Functional implications of genetic variation in non-coding DNA for disease susceptibility and gene regulation. Clin Sci (Lond) 104:493–501
2. Knight JC (2005) Regulatory polymorphisms underlying complex disease traits. J Mol Med (Berl) 83:97–109
3. Wang X, Tomso DJ, Liu X, Bell DA (2005) Single nucleotide polymorphism in transcriptional regulatory regions and expression of environmentally responsive genes. Toxicol Appl Pharmacol 207:84–90
4. Wang X, Tomso DJ, Chorley BN, Cho HY, Cheung VG, Kleeberger SR, Bell DA (2007) Identification of polymorphic antioxidant response elements in the human genome. Hum Mol Genet 16:1188–1200
5. Claessens F, Verrijdt G, Schoenmakers E, Haelens A, Peeters B, Verhoeven G, Rombauts W (2001) Selective DNA binding by the androgen receptor as a mechanism for hormone-specific gene regulation. J Steroid Biochem Mol Biol 76:23–30
6. Hsu MH, Savas U, Griffin KJ, Johnson EF (2007) Regulation of human cytochrome P450 4F2 expression by sterol regulatory element-binding protein and lovastatin. J Biol Chem 282: 5225–5236
7. Takai H, Araki S, Mezawa M, Kim DS, Li X, Yang L, Li Z, Wang Z, Nakayama Y, Ogata Y (2008) AP1 binding site is another target of FGF2 regulation of bone sialoprotein gene transcription. Gene 410:97–104
8. Buroker NE, Huang JY, Barboza J, Ledee DR, Eastman RJ Jr, Reinecke H, Ning XH, Bassuk JA, Portman MA (2012) The adaptor-related protein complex 2, alpha 2 subunit (AP2alpha2)

gene is a peroxisome proliferator-activated receptor cardiac target gene. Protein J 31:75–83
9. Huang CN, Huang SP, Pao JB, Hour TC, Chang TY, Lan YH, Lu TL, Lee HZ, Juang SH, Wu PP, Huang CY, Hsieh CJ, Bao BY (2012) Genetic polymorphisms in oestrogen receptor-binding sites affect clinical outcomes in patients with prostate cancer receiving androgen-deprivation therapy. J Intern Med 271:499–509
10. Huang CN, Huang SP, Pao JB, Chang TY, Lan YH, Lu TL, Lee HZ, Juang SH, Wu PP, Pu YS, Hsieh CJ, Bao BY (2012) Genetic polymorphisms in androgen receptor-binding sites predict survival in prostate cancer patients receiving androgen-deprivation therapy. Ann Oncol 23:707–713
11. Yu B, Lin H, Yang L, Chen K, Luo H, Liu J, Gao X, Xia X, Huang Z (2012) Genetic variation in the Nrf2 promoter associates with defective spermatogenesis in humans. J Mol Med (Berl) 90:1333–1342
12. Wu J, Richards MH, Huang J, Al-Harthi L, Xu X, Lin R, Xie F, Gibson AW, Edberg JC, Kimberly RP (2011) Human FasL gene is a target of beta-catenin/T-cell factor pathway and complex FasL haplotypes alter promoter functions. PLoS One 6:e26143
13. Alam M, Pravica V, Fryer AA, Hawkins CP, Hutchinson IV (2005) Novel polymorphism in the promoter region of the human nerve growth-factor gene. Int J Immunogenet 32:379–382
14. Rueda B, Gonzalez-Gay MA, Lopez-Nevot MA, Garcia A, Fernandez-Arquero M, Balsa A, Pablos JL, Pascual-Salcedo D, de la Concha EG, Gonzalez-Escribano MF, Martin J (2005) Analysis of vascular endothelial growth factor (VEGF) functional variants in rheumatoid arthritis. Hum Immunol 66:864–868
15. Rueda B, Perez-Armengol C, Lopez-Lopez S, Garcia-Porrua C, Martin J, Gonzalez-Gay MA (2006) Association between functional haplotypes of vascular endothelial growth factor and renal complications in Henoch–Schonlein purpura. J Rheumatol 33:69–73
16. Xie J, Yi L, Xu ZF, Mo XM, Hu YL, Wang DJ, Ren HZ, Han B, Wang Y, Yang C, Zhao YL, Shi DQ, Jiang YZ, Shen L, Qiao D, Chen SL, Yu BJ (2007) VEGF C-634G polymorphism is associated with protection from isolated ventricular septal defect: case-control and TDT studies. Eur J Hum Genet 15:1246–1251
17. Galecki P, Galecka E, Maes M, Orzechowska A, Berent D, Talarowska M, Bobinska K, Lewinski A, Bienkiewicz M, Szemraj J (2013) Vascular endothelial growth factor gene (VEGFA) polymorphisms may serve as prognostic factors for recurrent depressive disorder development. Prog Neuropsychopharmacol Biol Psychiatry 45:117–124
18. Cui QT, Li Y, Duan CH, Zhang W, Guo XL (2013) Further evidence for the contribution of the vascular endothelial growth factor gene in coronary artery disease susceptibility. Gene 521:217–221
19. Churchill AJ, Carter JG, Ramsden C, Turner SJ, Yeung A, Brenchley PE, Ray DW (2008) VEGF polymorphisms are associated with severity of diabetic retinopathy. Invest Ophthalmol Vis Sci 49:3611–3616
20. Yuan Q, Zuo X, Jia J (2009) Association between promoter polymorphisms of vascular endothelial growth factor gene and sporadic Alzheimer's disease among Northern Chinese Han. Neurosci Lett 457:133–136
21. Yang JW, Hutchinson IV, Shah T, Fang J, Min DI (2011) Gene polymorphism of vascular endothelial growth factor −1,154 G>A is associated with hypertensive nephropathy in a Hispanic population. Mol Biol Rep 38:2417–2425
22. Buroker NE, Ning XH, Zhou ZN, Li K, Cen WJ, Wu XF, Zhu WZ, Scott CR, Chen SH (2013) VEGFA SNPs and transcriptional factor binding sites associated with high altitude sickness in Han and Tibetan Chinese at the Qinghai-Tibetan Plateau. J Physiol Sci 63:183–193
23. Bryne JC, Valen E, Tang MH, Marstrand T, Winther O, da Piedade I, Krogh A, Lenhard B, Sandelin A (2008) JASPAR, the open access database of transcription factor-binding profiles: new content and tools in the 2008 update. Nucleic Acids Res 36:D102–D106
24. Sandelin A, Alkema W, Engstrom P, Wasserman WW, Lenhard B (2004) JASPAR: an open-access database for eukaryotic transcription factor binding profiles. Nucleic Acids Res 32:D91–D94
25. Sandelin A, Wasserman WW, Lenhard B (2004) ConSite: web-based prediction of regulatory elements using cross-species comparison. Nucleic Acids Res 32:W249–W252
26. Buroker NE, Ning XH, Zhou ZN, Li K, Cen WJ, Wu XF, Zhu WZ, Scott CR, Chen SH (2012) AKT3, ANGPTL4, eNOS3, and VEGFA associations with high altitude sickness in Han and Tibetan Chinese at the Qinghai-Tibetan Plateau. Int J Hematol 96:200–213
27. Rueda B, Lopez-Nevot MA, Lopez-Diaz MJ, Garcia-Porrua C, Martin J, Gonzalez-Gay MA (2005) A functional variant of vascular endothelial growth factor is associated with severe ischemic complications in giant cell arteritis. J Rheumatol 32:1737–1741
28. Lamani E, Wu Y, Dong J, Litaker MS, Acevedo AC, MacDougall M (2009) Tissue- and cell-specific alternative splicing of NFIC. Cells Tissues Organs 189:105–110
29. Pennisi E (2011) The biology of genomes. Disease risk links to gene regulation. Science 332:1031
30. Kumar V, Wijmenga C, Withoff S (2012) From genome-wide association studies to disease mechanisms: celiac disease as a model for autoimmune diseases. Semin Immunopathol 34:567–580
31. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA (2009) Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci USA 106:9362–9367
32. Kumar V, Westra HJ, Karjalainen J, Zhernakova DV, Esko T, Hrdlickova B, Almeida R, Zhernakova A, Reinmaa E, Vosa U, Hofker MH, Fehrmann RS, Fu J, Withoff S, Metspalu A, Franke L, Wijmenga C (2013) Human disease-associated genetic variation impacts large intergenic non-coding RNA expression. PLoS genetics 9:e1003201
33. Chorley BN, Wang X, Campbell MR, Pittman GS, Noureddine MA, Bell DA (2008) Discovery and verification of functional single nucleotide polymorphisms in regulatory genomic regions: current and developing technologies. Mutat Res 659:147–157
34. Prokunina L, Alarcon-Riquelme ME (2004) Regulatory SNPs in complex diseases: their identification and functional validation. Expert Rev Mol Med 6:1–15
35. Buckland PR (2006) The importance and identification of regulatory polymorphisms and their mechanisms of action. Biochim Biophys Acta 1762:17–28
36. Sadee W, Wang D, Papp AC, Pinsonneault JK, Smith RM, Moyer RA, Johnson AD (2011) Pharmacogenomics of the RNA world: structural RNA polymorphisms in drug therapy. Clin Pharmacol Ther 89:355–365