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VEGFA SNPs (rs34357231 & rs35569394), Transcriptional Factor Binding Sites and Human Disease

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Purpose: The human vascular endothelial growth factor (*VEGF)-A* gene transcribes a signaling protein involved in the regulation of angiogenesis, vasculogenesis and endothelial cell growth. Two insertion/deletion (I/D) simple nucleotide polymorphisms (SNPs, rs34357231 & rs35569394) in the promoter region of the gene have been significantly associated with several human diseases. These SNPs were computationally examined with respect to changes in punitive transcriptional factor binding sites (TFBS) and these changes were discussed in relation to the diseases.
Methods: The JASPAR CORE and ConSite databases were instrumental in identifying the TFBS. The Vector NTI Advance 11.5 computer program was employed in locating all the TFBS in the *VEGFA* gene from 2.7 kb upstream of the transcriptional start site to 1.6 kb past the 3'UTR. The JASPAR CORE database was also involved in computing each nucleotide occurrence (%) within the TFBS.
Results: Regulatory SNPs (rSNPs) in the promoter region of the *VEGFA* gene alter the DNA landscape for potential transcriptional factors (TFs) to attach resulting in changes in TFBS. The *VEGFA*-deletion (D) allele of these SNPs has been found to be a risk factor for diabetic

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retinopathy, diabetic microvascular complications in patients with type 1 diabetes mellitus, breast cancer in north Indian patients, and bladder cancer. The changes in TFs associated with the TFBS are examined with respect to these human diseases.

Conclusion: The *VEGFA*-insertion (I) allele provides punitive TFBS for the AR, EGR1 & 2, KLF5 and SP1 TFs whose BS do not occur with the *VEGFA*-D allele. These TFs have been linked to prostate cancer, cancer suppression and oncogenic processes and if not regulating the VEGFA gene may pose a risk for disease.

Keywords: VEGFA; rSNPs; TFBS; human disease.

1. INTRODUCTION

Simple nucleotide polymorphisms (SNPs) that affect gene expression by impacting gene regulatory sequences such as promoters, enhances, and silencers are known as regulatory SNPs (rSNPs) [1-4]. A rSNPs within a transcriptional factor binding site (TFBS) can change a transcriptional factor's (TF) ability to engage its binding site (BS) [5-8] in which case the TF would be unable to effectively regulate its target gene [9-13]. This concept is examined for the insertion/deletion (I/D) SNPs (rs34357231 & rs35569394) located at -2549bp from the transcriptional start site (TSS) of the vascular endothelial growth factor (VEGF)-A gene and their association with TFBS and human disease. The human *VEGFA* gene, a member of the PDGF/VEGF growth factor family, is encoded on chromosome 6 (6p21.3) and the transcribed protein is usually expressed as a 46-kDa disulfide-linked homodimer. Presently seven VEGF family members and 14 alternative splicing variants have been identified in humans [14-16]. Of the 14 splicing variants, 12 are VEGFA isoforms [16]. 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. This VEGFA (I/D) polymorphism has been associated with diabetic retinopathy [17], diabetic microvascular complications in patients with type 1 diabetes mellitus [18], breast cancer in north Indian patients [19], and bladder cancer risk [20], where the D allele in these studies has been considered the risk allele. In this report, the VEGFA I/D allelic associations with changes in potential TFBS and their possible relationship to the reported diseases or conditions is discussed. Some potential TFBS for these rSNPs have previously been discussed in association with high altitude sickness [21]. However, in this study a comprehensive examination is made of changes in TFBS created by the VEGFA I/D alleles and any changes associated with the above diseases.

2. METHODS

The JASPAR CORE database [22,23] and ConSite [24] 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 TFBS and rSNP location within the binding sites have previously been discussed [21,25-28]. The Vector NTI Advance 11.5 computer program (Invitrogen, Life Technologies) was used to locate the TFBS in the *VEGFA* gene (NCBI Ref Seq NM_001171626) from 2.7 kb upstream of the TSS to 1.6 kb past the 3'UTR which represents a total of 19.6 kbp.

3. RESULTS

3.1 VEGFA rSNPs (rs34357231 and rs35569394) and TFBS

The rs34357231 and rs35569394 rSNPs (I/D) are located -2551 and -2549 base pairs (bps), respectively, from the VEGFA TSS [21]. Sometimes the SNP alleles do not change the TFBS but in other instances each allele may provide a unique TFBS for a given TF such as shown in the Table 1. As an example, the rs34357231 VEGFA-I allele creates twelve punitive TFBS for the AR, EGR1 & 2, KLF5, MZFI_1-4, NFYB, NFATC2, NKX2-5 (var.2), NKX3-2, SP1 & 2, and STAT5a::STAT5b TFs which are involved with the gene regulation, transcription regulation, expression of cytokine genes in T-cells, transcription repression, regulation of cell growth, apoptosis, differentiation and immune responses, signal transduction and activation of transcription, respectively (Tables 1 and 2). The rs34357231 VEGFA D allele creates nine potential TFBS for

the HNF4α, HNF4γ, JUN, MYB, NFIC, NR2C2, NR4A2, PAX2 and RFX5 TFs which are involved with the expression of hepatic genes, TF activation, enhancers, hemopoietic progenitor cells, activating transcription and replication, repression of nuclear receptor signaling pathways, transcription regulation, kidney cell differentiation and transcription activation, respectively (Tables 1 and 2). There are eleven TFBS that are conserved between the two (I/D) alleles which are for the CREB1, ESR2, JUN:FOS, MEIS1, RUNX1 & 2, RXRα, TFAP2A & C, THAP1 and ZFX TFs, which are involved with the gene regulation, transcription regulation, expression of cytokine genes in T-cells, transcription repression, retinoic acid-mediated gene activation, regulation of cell growth, apoptosis, differentiation and immune responses, signal transduction and activation of transcription, respectively (Tables 1 and 2). Of these conserved TFBS, the CREB1 and ESR2 binding sites are lost when the rs35569394 VEGFA-D' allele is present (Table 1).

4. DISCUSSION

The D allele of these SNPs has been found to be at risk for diabetic retinopathy [17], diabetic microvascular complications in patients with type 1 diabetes mellitus [18], breast cancer in north Indian patients [19], and bladder cancer [20]. An important nuclear hormone receptor TFBS which is missing with VEGFA-D allele is for the androgen receptor but presence with the VEGFA-I allele (Tables 1 and 2). Androgen receptors are of the NR3C class of nuclear receptors which include mineralocorticoid, progesterone and glucocorticoid receptors which are expressed in bone marrow, mammary gland, prostate, testicular and muscle tissues. Androgen is critical for the development and maintenance of the male sexual phenotype and the androgen receptor has been linked to prostate cancer [29]. The early growth response 1 and 2 (EGR 1 and 2) TFBS are present with the VEGFA-I allele but not with the D allele. The EGR1 TF is a nuclear protein that functions as a transcriptional regulator and thought to be a cancer suppressor gene [30]. The Kruppel-like factor-5 (KLF5) and stimulating protein-1 & 2 (SP1 & 2) TFBS are present with the VEGFA-I allele but not with the D allele. The SP1/2 and KLF5 are part of a SP/KLF family of TFs which play a role in diverse cellular processes, including vascular smooth muscle cell (VSMC) proliferation, cell differentiation, apoptosis and oncogenic processes [31,32] and have previously been discussed with respect to the rs34357231 VEGFA SNP [33]. The myeloid zinc finger 1 (MZF1_1-4) TFBS occurs only with the presence of the VEGFA-I allele and its TF function as a transcription regulator that is involved with hematopoietic development (Tables 1 and 2). The nuclear factor of activated T-cells (NFATC2) TFBS also only occurs with the presence of the VEGFA-I allele and its TF is involved with induction of cytokine genes in T-cells (Tables 1 & 2). Other TFBS (NFYB, NKX2-5, NKX3-2 and STAT5a & b) which occur with the VEGFA-I allele and not the VEGFA-D allele have TFs involved with transcription machinery (Tables 1 and 2).

The hepatocyte nuclear factor 4 α and γ (HNF4α & γ) TFBS occur with the VEGFA-D allele and not the VEGFA-I allele have TFs that regulate the expression of several hepatic genes (Tables 1 and 2). The *jun* proto-oncogene (JUN) TFBS is only present with the VEGFA-D allele has a TF that promotes activity of NR5A1 leading to increased steroidogenic gene expression upon cAMP signaling pathway stimulation (Tables 1 and 2). The nuclear receptor subfamily 2, group C, member 2 (NR2C2) and nuclear receptor subfamily 4, group A, member 2 (NR4A2) TFBS also only occur with the VEGFA-D allele and have TFs that repress nuclear receptor signaling pathways such as retinoic acid receptor, retinoid X, vitamin D3 receptor, thyroid hormone receptor and estrogen receptor pathways as well as maintenance of meso-diencephalic dopaminergic neurons during development, respectively (Tables 1 and 2). The v-*myb* myeloblastosis viral oncogene homolog (MYB) TFBS that only occurs with the VEGFA-D allele has a potential binding site for its TF that plays an important role in the control of proliferation and differentiation of hematopoietic progenitor cells (Tables 1 and 2). The paired box gene 2 (PAX2) TFBS that occurs only with the VEGFA-D allele has a binding site for its TF that may have a role in kidney cell differentiation (Tables 1 and 2). The regulatory factor X, 5 (RFX5) TFBS that also only occurs with the VEGFA-D allele is a binding site for its TF that activates transcription from class II MHC promoters (Tables 1 and 2).

Table 1. The VEGFA-18bp I/D SNPs (rs34357231 & rs35569394) that were examined in this study where the minor allele is in red. Also listed are the transcriptional factors (TF), their potential binding sites (TFBS) containing these SNPs and DNA strand orientation. TFs in red differ between the SNP alleles while the TF in green have no binding sites in rs35569394. Where upper case nucleotide designates the 90% conserved BS region. Also listed are the numbers (#) of binding sites in the gene for the given TF.
Note: TFs can bind to more than one nucleotide sequence

SNP	Allele	TFs	Protein name	# of Sites	TFBS	Strand
rs34357231	I	AR	Androgen receptor	1	ggGacCAgtcaGtct	minus
18bp I/D	0.28					
		CREB1	cAMP responsive element binding protein 1	4	tGAgGcct	minus
		EGR1	Early growth response 1	1	actCttCCcaCagg	plus
		EGR2	Early growth response 2	1	actcttCcCaCaggc	minus
		ESR2	Estrogen receptor 2 (ER beta)	1	ggGgctctgaggcct	minus
		JUN:FOS	*Jun* proto-oncogene FBJ murine osteosarcoma viral oncogene homolog	7	Tgactgg	plus
		KLF5	Kruppel-like factor 5 (intestinal)	1	gtccCaCtCt	plus
		KLF5	Kruppel-like factor 5 (intestinal)	1	actcttCCCa	plus
		Meis1	Meis homeobox 1	1	acCaGTCAgtctgat	minus
		MZF1_1-4	Myeloid zinc finger 1	32	tGGGaA	minus
		MZF1_1-4	Myeloid zinc finger 1	21	gtGGGA	minus
		MZF1_1-4	Myeloid zinc finger 1	21	gtGGGA	minus
		NFYB	Nuclear transcription factor Y, beta	1	agtgggaCCAgTcag	minus
		NFATC2	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	10	tcTTCCc	plus
		NKX2-5 (var.2)	NK2 homeobox *5*	1	tccCaCTCttc	plus
		NKX3-2	NK3 homeobox *2*	1	aagAGTggg	minus
		RUNX1	Runt-related transcription factor 1	1	gccTGtGGgaa	minus
		RUNX1	Runt-related transcription factor 1	1	ctcTGaGGcct	minus
		RUNX2	Runt-related transcription factor 2	1	gaggccTGTGGgaag	minus
		RUNX2	Runt-related transcription factor 2	1	gggctcTGaGGcctg	minus
		RXRa	Retinoid X receptor, alpha	1	ctgAGgcCtgt	minus
		SP1	Specificity Protein 1	1	actCttCCcac	plus
		SP1	Specificity Protein 1	1	gtcCCaCtctt	plus
		SP2	Specificity Protein 2	1	gtcCCaCtcttccca	plus
		STAT5a::STAT5b	Signal transducer and activator of	1	tcTTCccacAg	plus

SNP	Allele	TFs	Protein name	# of Sites	TFBS	Strand
			transcription 5A and transcription 5B			
		TFAP2A	Transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	1	tcttcCCacaGGcct	plus
		TFAP2A	Transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	1	tgaggCCtgtGGgaa	minus
		TFAP2C	Transcription factor AP-2 gamma (activating enhancer binding protein 2 gamma)	1	tcttcCcacaGGcct	plus
		TFAP2C	Transcription factor AP-2 gamma (activating enhancer binding protein 2 gamma)	1	tgaggCctgtGGgaa	minus
		TFAP2C	Transcription factor AP-2 gamma (activating enhancer binding protein 2 gamma)	1	acaggCctcaGagcc	plus
		THAP1	THAP domain containing, apoptosis associated protein 1	2	cttCCcaca	plus
		ZFX	Zinc finger protein, X-linked	1	ggctctgaGGCCTg	minus
	D	CREB1	cAMP responsive element binding protein 1	3	tGAgGcca	minus
		ESR2	Estrogen receptor 2 (ER beta)	1	agGccAgtcagtctg	minus
		HNF4a	hepatocyte nuclear factor 4 □	1	ctggcctcagagccc	plus
		HNF4g	hepatocyte nuclear factor 4 □	1	ggggctCTGAGgcca	minus
		JUN	*jun* proto-oncogene	1	gctcTGAgGccAg	minus
		JUN::FOS	*Jun* proto-oncogene FBJ murine osteosarcoma viral oncogene homolog	7	TgActgg	plus
		Meis1	Meis homeobox 1	1	gcCaGTCAgtctgat	minus
		MYB	v-*myb* myeloblastosis viral oncogene homolog	1	cagACTGaCt	plus
		NFIC	Nuclear factor 1 C-type	41	cTGGcc	plus
		NR2C2	Nuclear receptor subfamily 2, group C, member 2	1	gggGctctgaGgcca	minus
		NR4A2	Nuclear receptor subfamily 4, group A, member 2	5	gAGgcCAg	minus
		PAX2	Paired box gene 2	1	agtCagtc	minus
		RFX5	Regulatory factor X, 5 (influences HLA class II expression)	1	ctgacygGcctCaga	plus
		RUNX1	Runt-related transcription factor 1	1	ctcTGaGGcca	minus
		RUNX2	Runt-related transcription factor 2	1	gggctcTGaGGccag	minus
		RXRa	Retinoid X receptor, alpha	1	ctgAGgcCagt	minus
		TFAP2A	Transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	1	actggCCtcaGagcc	plus

SNP	Allele	TFs	Protein name	# of Sites	TFBS	Strand
		TFAP2C	Transcription factor AP-2 gamma (activating enhancer binding protein 2 gamma)	1	actggCctcaGagcc	plus
		THAP1	THAP domain containing, apoptosis associated protein 1	2	ctggCctca	plus
		ZFX	Zinc finger protein, X-linked	1	ggctctgaGGCCag	minus
rs35569394	D'	CREB1	cAMP responsive element binding protein 1		missing in rs34357231 (D)	
		ESR2	Estrogen receptor 2 (ER beta)		missing in rs34357231 (D)	

Table 2. Transcriptional factor (TF) descriptions

TFs	TF description
AR	Androgen receptors (ARs) (also known as dihydrotestosterone receptors) are nuclear hormone receptors of the NR3C class, which also includes mineralocorticoid, progesterone and glucocorticoid receptors. They are expressed in bone marrow, mammary gland, prostate, testicular and muscle tissues where they exist as dimers coupled to Hsp90 and HMGB proteins, which are shed upon ligand binding. Activated androgen receptors bind to nuclear response elements of the genome, with an inverted palindromic 15 nucleotide sequence, to regulate gene transcription.
CREB1	Phosphorylation-dependent transcription factor that stimulates transcription upon binding to the DNA cAMP response element (CRE), a sequence present in many viral and cellular promoters. Transcription activation is enhanced by the TORC coactivators which act independently of Ser-133 phosphorylation. Involved in different cellular processes including the synchronization of circadian rhythmicity and the differentiation of adipose cells
EGR1	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 differentitation and mitogenesis. Studies suggest this is a cancer suppresor gene.
EGR2	The protein encoded by this gene is a transcription factor with three tandem C2H2-type zinc fingers. Sequence-specific DNA-binding transcription factor. Binds to two specific DNA sites located in the promoter region of HOXA4
ESR2	Nuclear hormone receptor. Binds estrogens with an affinity similar to that of ESR1, and activates expression of reporter genes containing estrogen response elements (ERE) in an estrogen-dependent manner. Estrogen controls many cellular processes including growth, differentiation and function of the reproductive system. In females, estrogen's main targets are the ovaries, uterus, vagina and mammary glands. In the male, target organs are the testes, prostate and epididymis. Estrogen is also responsible for the growth and maintenance of the skeleton and the normal functioning of the cardiovascular and nervous systems
HNF4a	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
HNF4g	Transcription factor. Has a lower transcription activation potential than HNF4-alpha Go annotations related to this gene include steroid

TFs	TF description
	hormone receptor activity and sequence-specific DNA binding transcription factor activity. An important paralog of this gene is RXRa
JUN	Transcription factor that recognizes and binds to the enhancer heptamer motif 5'-TGA[CG]TCA-3'. Promotes activity of NR5A1 when phosphorylated by HIPK3 leading to increased steroidogenic gene expression upon cAMP signaling pathway stimulation
JUN:FOS	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.
KLF5	Transcription factor that binds to GC box promoter elements. Activates transcription of genes.
MEIS1	Homeobox genes, of which the most well-characterized category is represented by the HOX genes, play a crucial role in normal development.
MZF1_1-4	Binds to target promoter DNA and functions as trancription regulator. May be one regulator of transcriptional events during hemopoietic development. Isoforms of this protein have been shown to exist at protein level.
MYB	This gene encodes a transcription factor that is a member of the MYB family of transcription factor genes. Plays an important role in the control of proliferation and differentiation of hematopoietic progenitor cells.
NFIC	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.
NFYB	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.
NFATC2	Plays a role in the inducible expression of cytokine genes in T-cells, especially in the induction of the IL-2, IL-3, IL-4, TNF-alpha or GM-CSF. Promotes invasive migration through the activation of GPC6 expression and WNT5A signaling pathway.
NKX2-5 (var.2)	This gene encodes a member of the NK family of homeobox-containing proteins. Transcriptional repressor that acts as a negative regulator of chondrocyte maturation.
NKX3-2	This gene encodes a member of the NK family of homeobox-containing proteins. Transcriptional repressor that acts as a negative regulator of chondrocyte maturation.
NR2C2	Members of the nuclear hormone receptor family, such as NR2C2, act as ligand-activated transcription factors 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.
NR4A2	Transcriptional regulator which is important for the differentiation and maintenance of meso-diencephalic dopaminergic (mdDA) neurons during development.
PAX2	Probable transcription factor that may have a role in kidney cell differentiation.
PBX1	Activates insulin, somatostatin, glucokinase, islet amyloid polypeptide and glucose transporter type 2 gene transcription. Particularly involved in glucose-dependent regulation of insulin gene transcription.
RFX5	Activates transcription from class II MHC promoters. Recognizes X-boxes.

TFs	TF description
RUNX1	Core binding factor (CBF) is a 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 CBF and is thought to be involved in the development of normal hematopoiesis.
RUNX2	Transcription factor involved in osteoblastic differentiation and skeletal morphogenesis. Essential for the maturation of osteoblasts and both intramembranous and endochondral ossification.
RXRA	Retinoid X receptors (RXRs) and retinoic acid receptors (RARs), are nuclear receptors that mediate the biological effects of retinoids by their involvement in retinoic acid-mediated gene activation.
SP1	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	Binds to GC box promoters elements and selectively activates mRNA synthesis from genes that contain functional recognition sites.
STAT5A:STAT5B	Carries out a dual function: signal transduction and activation of transcription. Regulates the expression of milk proteins during lactation.
TFAP2□	The protein encoded by this gene is a transcription factor that binds the consensus sequence 5'-GCCNNNGGC-3' and activates the transcription of some genes while inhibiting the transcription of others.
TFAP2C	Sequence-specific DNA-binding protein that interacts with inducible viral and cellular enhancer elements to regulate transcription of selected genes. AP-2 factors bind to the consensus sequence 5'-GCCNNNGGC-3' and activate genes involved in a large spectrum of important biological functions including proper eye, face, body wall, limb and neural tube development.
THAP1	DNA-binding transcription regulator that regulates endothelial cell proliferation and G1/S cell-cycle progression.
ZFX	A member of the krueppel C2H2-type zinc-finger protein family and probable transcriptional activator.

TFBS that are available with the VEGFA-I allele and not with the D allele could be responsible for the diseases listed above a risk factor with presence of a D allele. TFBS associated with the VEGFA-I allele and not present with the D allele that should be of concern would be for the AR, EGR1 & 2, KLF5 and SP1 TFs. Each or all of these TFs whose binding site is missing with the VEGFA-D allele could put a person at risk for diabetes and cancer. 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 the insertion/deletion (I/D) rSNPs (rs34357231 & rs35569394) can provide different TFBS which in turn are also significantly associated with human disease.

5. CONCLUSION

In this study, examples of punitive alterations in TFBS by the rs34357231 and rs35569394 rSNPs result in different BS for TFs that regulate the *VEGFA* gene. Since the rSNPs *VEGFA*-D allele has previously been found to be a risk for diabetic retinopathy, diabetic microvascular complications in patients with type 1 diabetes mellitus, breast cancer in north Indian patients, and bladder cancer, this study identifies possible reasons for that risk. The reasons are attributed to TFBS changes resulting from the rSNPs that would cause different TFs regulation of the gene with the *VEGFA*-I allele than with the *VEGFA*-D allele. The *VEGFA*-I allele provides punitive TFBS for the AR, EGR1 & 2, KLF5 and SP1 TFs that do not occur with the *VEGFA*-D allele. These TFs have been linked to prostate cancer and oncogenic processes and if not regulating the VEGFA gene may pose a risk for disease. The focus of this work has been to draw attention to punitive TFBS alterations created by the rSNPs; however, further investigation by protein/DNA binding electrophoretic mobility shift assays and lucerifase gene activation studies need to be conducted which is beyond the scope of this initial evaluation of these VEGFA SNPs and TFBS.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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