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Regulatory SNPs and transcription factor binding sites in *ADRBK1*, *AKT3*, *ATF3*, *DIO2*, *TBXA2R* and *VEGFA*

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Regulatory single nucleotide polymorphisms, which change the DNA binding sites for transcription factors, were reviewed for the *ADRBK1* (*GRK2*), *AKT3*, *ATF3*, *DIO2*, *TBXA2R,* and *VEGFA* genes. Changes in transcription factor binding sites within these genes may result in human sickness and disease.

Introduction

Genome-wide association studies have identified nearly 6,500 disease- or trait-predisposing single nucleotide polymorphisms (SNPs) over the last decade. Only 7% of these SNPs are located in protein-coding regions of the genome,[1,2] with the remaining 93% being located within non-coding areas,[3,4] such as regulatory or intergenic regions. SNPs that occur in the putative regulatory region of a gene in which a single base change in the putative transcription factor DNA binding site (TFBS) may affect the process of gene expression are drawing more attention.[5-7] An SNP in a TFBS can have multiple consequences. Often, the SNP does not change the interaction with the transcription factor (TF) nor does it alter gene expression, since TFs will usually recognize a number of different binding sites in the gene. However, in some allele-specific gene expression cases, the binding affinity of the TF to a response element may be altered, with the SNP increasing or decreasing the TF binding ability. In rare cases, an SNP may eliminate the TF natural binding site or generate a new binding site. In such cases, the gene is no longer regulated by the original TF. Therefore, functional regulatory SNPs (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 on the topic.[7-10] In this review, rSNPs will be considered as SNPs that alter the DNA landscape for TF binding, resulting in different TFBS that may affect gene regulation.

In genetics, linkage disequilibrium (LD) is defined as the non-random association, in a given population, between alleles of 2 or more loci.[11] LD between SNPs in the regulatory region of a gene can be used as a method for identifying associations of certain haplotypes with sickness or disease in a population.[12,13] This can be achieved when levels of LD between SNPs within haplotypes are seen to change substantially in a disease or sickness population group when compared to the normal baseline population. In such cases, the relationship between LD, SNPs, and TFBS can be used to identify potential gene regulatory TF binding changes, which could result in disease or sickness.[14-21] In this report, LD will be considered as the non-random association of SNP alleles within the gene. The purpose of this review is to highlight some of these genetic associations for 6 genes involved in known human diseases and sicknesses that have been recently studied.[14,16-19,22,23]

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ADRBK1 (*GRK2*) rSNPs and Heart Disease

G protein-coupled receptor kinases (GRKs) are a family of 7 serine/threonine protein kinases with important and varied roles in regulating cellular signaling.[24-26] The *GRK2* gene (*ADRBK1*), which is located in chromosome 11q13.1 and is about 20 kb long, is an important regulator of β-adrenergic signaling and plays a central role in heart failure pathology.[27-29] Consequently, the inhibition of GRK2 has become an emerging treatment option for heart failure, since inhibition appears to be a powerful therapeutic approach and provide complementation to β-blockade.[28] When the heart is injured or stressed, the sympathetic nervous system is activated and adrenergic overdrive occurs via an excess of catecholamines, which leads to the uncoupling and desensitization of β-adrenergic receptors (β ARs) in the heart, brought about by the up-regulation of GRK2.[30-32] Lymphocyte GRK2 levels increase during acute myocardial infarction and are associated with worsening of the cardiac function.[33] Enhanced GRK2 activity has been shown to contribute to cardiac decompensation.[34,35] Independently of its uncoupling properties on β ARs, GRK2 appears to be a pro-death kinase, especially in stressed myocytes.[36,37] Consequently, inhibition of GRK2 or its genetic ablation has been shown to improve cardiac function in myocardial injury.[38]

rSNPs in the promoter region (rs12286664; -703 bp), introns (rs1894111, rs7128315, and rs948988) and 3′ UTR (rs4370946) of the *ADRBK1* gene are in LD in the black population.[27] These rSNP alleles alter the DNA landscape for potential TF binding, resulting in changes in TFBS. The alleles of each rSNP were found

to produce unique TFBS, resulting in potential changes in *ADRBK1* regulation.[18] As an example, the rs7128315 rSNP common *ADRBK1-G* allele creates TFBS for SREBF1 and 2, two TFs that are involved in the cholesterol synthesis pathway. These TFBS occur only once in the gene and do not occur when the rs7128315 rSNP minor *ADRBK1-A* allele (0.32) is present.[18] SREBF1 and 2 are master regulators of lipid homeostasis[39,40] and have been associated with coronary heart disease in Han Chinese[41] and heart dysfunction.[42]

AKT3 rSNPs and High Altitude Sickness

The phosphatidylinositol 3-kinase (PI3K)/AKT pathway plays a key role in numerous cellular functions, including proliferation, adhesion, migration, invasion, metabolism, survival[43], and angiogenesis. The PI3K/AKT pathway modulates the expression of angiogenic factors, such as nitric oxide and angiopoietins.[44] *V-AKT murine thymoma viral oncogene homolog 3* (*AKT3*) is located in chromosome 1q44 and is about 362.8 kb long. *AKT3* is one of 3 isoforms of the AKTs, which are major downstream targets of growth factor receptor tyrosine kinases that signal through PI3K.[45] The *AKT3* rSNP in intron 1 (rs4590656) was recently found to be significantly associated with 3 physiological parameters (hemoglobin, hematocrit, and red blood cell count) in chronic mountain sickness patients, indicating the strong association of this gene with angiogenesis.[46] The rs4590656 rSNP common *AKT3-C* allele creates TFBS for the ARNT:AHR and HIF1α:ARNT TFs, which are involved in xenobiotic metabolism and cellular and systemic responses to hypoxia, respectively. These TFBS are not present in the rs4590656 rSNP minor *AKT3-T* allele (0.41).[16] Other *AKT3* rSNPs in intron 1 (rs10157763, rs10927067, and rs2125230) have been significantly associated with aggressive prostate cancer (PCa).[47] These rSNPs also alter the TFBS for TFs that regulate the *AKT3* gene,[16] where the rs2125230 rSNP minor *AKT3-A* allele (0.26) creates a unique TFBS for the IRF1 TF.[16] The IRF1 (interferon regulatory factor 1) TF is a member of the interferon regulatory transcription factor (IRF) family. Members of this family share several common effects, which include their activity as antiviral agents that fight tumors. Other rSNPs in intron 1 (rs4132509, rs12031994, and rs2345994) have been found to be significantly associated with renal cell carcinoma risk.[48] The rs12031994 rSNP minor *AKT3-A* allele (0.13) creates a unique TFBS that occurs only once in the gene for the TAL1:GATA1 TF, which has been implicated in the genesis of hematopoietic malignancies.[49] All 7 *AKT3* rSNPs have been found to alter the DNA landscape resulting in changes in TFBS.[16]

ATF3 rSNPs and Hypospadias

The *activating transcription factor 3* (*ATF3*) gene is located in chromosome 1q32.3 and is about 55.4 kb long. It is a member of the activating transcription factor/cAMP responsive element binding (CREB) protein family of transcription factors, which share the basic region-leucine zipper (bZip) DNA binding motif (TGACGTCA). This gene is induced by a variety of signals, including many of those encountered by cancer cells, and is involved in the complex process of cellular stress response.[50-52] *ATF3* has been viewed as a hub of the cellular adaptive response network, which allows cells to adapt to disturbances in homeostasis.[53] This gene has been shown to be up-regulated during sexual differentiation,[54] which indicates a potential role in hypospadias. Three unlinked *ATF3* rSNPs (rs3125289, rs1877474, and rs11119982), which span a 16 kb region of intron 1, have been independently found to be significantly associated with the risk of hypospadias.[55] These rSNP alleles alter the DNA landscape, resulting in changes in TFBS. These TFBS changes have been examined with respect to the human etiology of hypospadias, which have been found to be significantly associated with the rSNPs.[17]

The rs3125289 rSNP *AFT3-T* allele (0.55) creates a unique TFBS for the SRY TF, which is a transcriptional regulator that controls a genetic switch in male development. The TFBS for the SRY TF is only found once in the *ATF3* gene and does not occur in the rs3125289 rSNP minor *AFT3-C* allele.[17] The rs11119982 rSNP *AFT3-T* allele (0.51) creates a unique TFBS for the MYB TF, which plays an important role in the control of proliferation and differentiation of haematopoietic progenitor cells. The TFBS for the MYB TF is only found once in the *ATF3* gene and does not occur in the rs11119982 rSNP minor *AFT3-C* allele.[17]

DIO2 rSNPs and Type 2 Diabetes Mellitus

Type 2 deiodinase (*DIO2*) gene encodes a deiodinase that converts the thyroid pro-hormone thyroxine (T4) into the biologically active triiodothyronine (T3), which plays an important role in the regulation of energy balance and glucose metabolism.[56-59] DIO2 is found in the thyroid gland, cardiac and skeletal muscle, brown adipose tissue, placenta, pituitary, central nervous system, and, at low levels, in kidney and pancreas.[60-62] The *DIO2* gene maps to human chromosome 14q24.3 and is about 15 kb long. The coding region consists of 2 exons separated by an intron of approximately 7.4 kb.[63] Several SNPs have been found in the gene that have been studied in association with mental retardation,[64] osteoarthritis,[65] and early-onset type 2 diabetes mellitus (T2DM).[66] Three of the common SNPs in the gene (rs225014, rs225012, and rs225010) have been found to be in strong LD with each other, while the rs225012 and rs225010 SNPs have been shown to have a positive association with mental retardation.[64] The haplotypes of 2 SNPs (rs225014 and rs12885300) have been shown to have a significant association with symptomatic osteoarthritis in Dutch women.[65] Three SNPs (rs225011, rs225014, and rs225015), which are in LD, were found to be modestly associated with early-onset of T2DM in Pima Indians, while these SNPs and rs6574549 were found to be nominally associated with hepatic glucose output.[66] The rs6574549 SNP was also found to be associated with insulin fasting, insulin action, and energy expenditure.[66] These studies suggest that some *DIO2* SNPs may affect the regulatory network for its

expression in humans. A recent study has reported that association between *DIO2* SNPs in LD and TFBS changes occur.[23] rSNPs were found in the promoter region involving a novel SNP in the 5′UTR (−2035bp) of *DIO2* gene (rs12885300), intron 1 (rs225010, rs225011, and rs225012), exon 2 [rs225014 (Thr92Ala)] and 3′ UTR (rs6574549 and rs225015), all of which are in LD.[66] The rs225012 rSNP common *DIO2-G* allele creates 4 unique TFBS for the E2F6, ELF1, EGR1, and SPI1 TFs, which occur only once in the *DIO2* gene and are involved in the control of the cell cycle and action of tumor suppressor proteins, transcription regulation, and gene expression during myeloid and B-lymphoid cell development, respectively.[23] The rs225012 rSNP minor *DIO2-A* allele (0.29) creates 2 unique TFBS for the HOXA5 and NKX3–2 TFs, which occur only once in the *DIO2* gene and are involved in the development regulatory system and negative regulation, respectively.[23] The rs225014 rSNP common *DIO2-T* allele (0.82) creates a unique TFBS for the FOXC1 TF, which occurs only once in the *DIO2* gene and is an important regulator of cell viability and resistance to oxidative stress.[23] The rs6574549 rSNP common *DIO2-G* allele creates a unique TFBS for the LHX3 TF, which occurs only once in the *DIO2* gene and is required for pituitary development and motor neuron specification.[23] The rs6574549 rSNP rare *DIO2-G* allele (0.06) creates a unique TFBS for the POU2F2 TF, which occurs only once in the *DIO2* gene and regulates transcription in a number of tissues in addition to activating immunoglobulin gene expression.[23] The rs225015 rSNP common *DIO2-G* allele creates a unique TFBS for the EBF1 TF, which occurs only once in the *DIO2* gene and is a transcriptional activator.[23] The rs225015 rSNP minor *DIO2-A* allele (0.40) creates a unique TFBS for the TCF7L2 TF, which occurs only once in the *DIO2* gene and is implicated in blood glucose homeostasis.[23]

TBXA2R rSNPs and Asthma

The *thromboxane A2 receptor* (*TBXA2R*) gene is located in chromosome 19p13.3 and is a member of the 7-transmembrane G-protein-coupled receptor super family, which interacts with intracellular G proteins. It regulates different downstream signaling cascades and induces many cellular responses, including intracellular calcium influx, cell migration and proliferation, and apoptosis.[67] This gene is abundantly expressed in different tissues at the mRNA and protein levels), including erythroleukemia cells, vascular and bronchial smooth muscle, uterus and placental tissue, endothelium, epithelium, trophoblasts, thymus, liver, and small intestine, and is targeted by the TBXA2R ligand thromboxane A2 (TXA2).[68] The activation of TBXA2R in bronchial smooth muscle cells by its ligand results in intercellular calcium mobilization with subsequent bronchoconstriction. This contributes to bronchial smooth muscle hyperplasia and airway remodeling, which occurs in response to chronic airway inflammation in asthma.[69]

Four linked *TBXA2R* rSNPs (rs2238631, rs2238632, rs2238633 and rs2238634), which span a 431 bp region of intron 1 have been found to be in LD with 2 exon 3 SNPs [rs11318632 (c.795 T > C) and rs4523 (c.924 T > C)],[70] which are approximately 8.4 kb downstream from the rSNPs in intron 1. The rs11318632 and rs4523 rSNPs from exon 3 are synonymous changes and unlikely to influence the characteristics of the receptor protein. The exon 3 rSNPs have been associated with asthma and its related phenotypes in Asian populations. The rs4523 SNP has been associated with adult asthma in a Japanese population[71] and with childhood atopic asthma in a Chinese population.[72] The rs11318632 SNP has been found to be associated with atopic asthma in a Korean population.[73] Two haplotypes (H2 and H4) involving the 4 linked *TBXA2R* SNPs from intron 1 where found to influence *TBXA2R* transcriptional activity and were also associated with asthma-related phenotypes.[70]

Four rSNPs (rs2238631, rs2238632, rs2238633, and rs2238634) in intron one, 2 rSNPs (rs1131882 and rs4523) in exon 3 and one rSNP (rs5756) in the 3′UTR of the *TBXA2R* gene have been associated with childhood-onset asthma in Asians. These rSNP alleles alter the DNA landscape resulting potential TFBS changes. These TFBS changes were examined with respect to asthma, which has been found to be significantly associated with the rSNPs.

The rs2238631 rSNP common *TBXA2R-G* allele creates a unique TFBS for the FOXC1 TF, which occurs only once in the *TBXA2R* gene and is an important regulator of cell viability and resistance to oxidative stress.[19] The rs2238631 rSNP minor *TBXA2R-A* allele (0.18) creates 2 unique TFBS for the ELK1 and ELK4 TFs, which each occur only once in the *TBXA2R* gene and are involved in the ras-raf-MAPK signaling cascade, transcriptional activation, and repression, respectively.[19] The rs2238632 rSNP minor *TBXA2R-T* allele (0.49) creates 2 unique TFBS for the CREB1 and HIF1α:ARNT TFs, which each occur only once in the gene and are involved in DNA binding, cellular, and systemic responses to hypoxia, respectively.[19] The rs2238634 rSNP minor TBXA2R-T allele (0.19) creates a unique TFBS for the HLTF TF which occurs only once in the TBXA2R gene and whose protein alters the chromatin structure around certain genes during transcription.[19] The rs1131882 rSNP common *TBXA2R-T* allele (0.57) creates a unique TFBS for the SOX17 TF, which occurs only once in the gene and is a transcription regulator that binds target promoter DNA and bends the DNA.[19] The rs4523 rSNP minor *TBXA2R-C* allele (0.21) creates a unique TFBS for the AR TF, which occurs only once in the gene and is a steroid hormone receptor that regulates eukaryotic gene expression and affects cellular proliferation and differentiation in target tissues.[19]

VEGFA rSNPs and Hypoxia

The vascular endothelial growth factor (VEGF) is a family of key regulators of critical physiological and pathological angiogenesis,[74] including tissue growth, wound healing, rheumatoid arthritis, proliferative retinopathies, cardiovascular disease, and cancer.[75] VEGF is a growth factor activator for angiogenesis, vasculogenesis, and endothelial cell growth. VEGF is an important component of the pathogenesis of

high altitude adaptation and sickness, as shown is several studies,[76-81] but not in others.[82] Presently, 7 *VEGF* family members and 14 alternative splicing variants have been identified in humans.[83-85] Of the 14 splicing variants, 12 are *VEGFA* isoforms,[85] with 3 (*VEGFA-121*, *-165* and *-189*) being differentially expressed in humans visiting or living in high altitude environments, as well as in chronic mountain sickness patients.[77,78] Among all family members, *VEGFA*, which is located in chromosome 6p12, is the most potent and best known angiogenic protein and exerts its biologic effect through its interaction with cell-surface receptors, which triggers a cascade of downstream dimerizations and phosphorylations.[86]

Mountain sickness occurs among humans visiting or inhabiting high altitude environments. Genetic analyses of 7 rSNPs in the promoter region of *VEGFA* gene were analyzed in lowland (Han) and highland (Tibetan) Chinese.[14] The 7 *VEGFA* rSNPs were evaluated in Han and Tibetan patients with acute and chronic mountain sickness, respectively. The rSNPs (rs699947, rs34357231, rs79469752, rs13207351, rs28357093, rs1570360, and rs2010963) are found in the promoter region, −2578 bps to −634 bps from the transcriptional start site. These rSNPs are found in TFBS and all alter these binding sites.[14] Arterial oxygen saturation of hemoglobin (SaO_2) has been found to be significantly associated with the rs699947, rs34357231, rs13207351, and rs1570360 rSNPs in Han patients with acute mountain sickness.[14] Mountain sickness was found to be significantly associated with these rSNPs when compared to their Han and Tibetan control groups, indicating that these nucleotide substitutions result in TFBS changes that apparently have a physiological effect in the development of high altitude sickness.[14] LD was found between rSNPs rs13207351 and rs1570360, and between rSNPs rs79469752 and rs28357093, in control and sickness groups.[14,87] The binding site for the HIF1α and ARNT TFs is generated by the rs699947 common rSNP *VEGFA-C* allele, but not by the minor *VEGFA-A* allele (0.23). This rSNP is not in LD with the other rSNPs in the *VEGFA* promoter.[14] The HIF1αand ARNT TFs play an essential role in cellular and systemic responses to hypoxia and their binding site occurs only in the promoter of the *VEGFA* gene.[14] Consequently, the rs699947 rSNP may have an impact on SaO_2 in patients with high altitude mountain sickness.

SNPs, Transcription Factor Binding Sites, and Ethnic Populations

A given rSNP allele frequency can vary between ethnic or racial groups due to historical population bottlenecks. This would affect the occurrence of TFBSs and TFs and should impact the groups that are susceptible to disease or sickness. As an example, the rs225015 rSNP *DIO2-A* allele creates a TFBS for the TCF7L2 TF, which is implicated in blood glucose homeostasis and has an allele frequency of 0.40 in most ethnic and racial groups; however, the frequency is 0.81 in Pima Indians.[66] This rSNP is modestly associated with early-onset T2DM and hepatic glucose output in the Pima Indians of Arizona,[66] which may be in part the result of the higher occurrence of the *DIO2-A* allele and the TCF7L2 binding site than in most of the other ethnic or racial groups.

Concluding Remarks

In summary, SNPs occurring in the non-coding regions of the reviewed genes have been found to be associated with human diseases or sicknesses. These non-coding regions host the binding sites for transcription factors that regulate gene expression. The rs7128315 *ADRBK1* rSNP, which has been associated with coronary heart disease and dysfunction in Asians alters the SREBF1 and 2 binding sites and could therefore interfere with lipid homeostasis. The rs2125230 *AKT3* rSNP, which has been associated with aggressive prostate cancer, alters the IRF1 binding site and could interfere with antiviral agents that fight tumors. The rs3125289 *AFT3* rSNP, which has been associated with the risk of hypospadias, alters the SRY binding site, which could affect male development. The rs225015 *DIO2* rSNP, which is modestly associated with early-onset of T2DM in Pima Indians, alters the TCF7L2 binding site, which is implicated in blood glucose homeostasis. The rs4523 *TBXA2R* rSNP, which has been associated with asthma in Asians, alters the AR binding site, which could affect cellular proliferation and differentiation. The rs699947 *VEGFA* rSNP, which has been associated with arterial oxygen saturation of hemoglobin, alters the HIF1α:ARNT binding site, which could affect the cellular and systemic responses to hypoxia. In conclusion, regulatory SNPs underlying the allele-specific changes in transcription factor binding sites can alter the regulation of these genes.

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