Editorial

Gene Team in Blood Pressure Genetics

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Renin–angiotensin system (RAS) genes were the first cab off the rank when the search for the molecular genetic basis of hypertension began 3 decades ago.1,2 Despite an enormous amount of research over the years, no consistent evidence has, however, emerged to convincingly implicate any of the RAS genes in essential hypertension etiology.

See Article by Scurrah et al

In the current issue, Scurrah et al3 report findings from a comprehensive study examining association of systolic blood pressure (SBP) with 88 tagging single nucleotide polymorphisms (SNPs) in and near the renin gene (REN), angiotensinogen gene (AGT), angiotensin-converting enzyme gene (ACE), angiotensin II type 1 receptor gene (AGTR1), and aldosterone synthase gene (CYP11B2) in 2872 individuals from 809 pedigrees in the Victorian Family Heart Study.1 Two SNPs at the ACE locus and 1 at AGTR1 were associated with 1.7 to 2.5 mm Hg lower SBP. The researchers also examined sex differences, finding 2 SNPs at the AGT locus and 1 at ACE were associated with SBP in males, as was the case for a different SNP at ACE in females. They further noted interactions between another 14 individual SNPs and SBP. Of these, 2 SNP pairs were at REN, 1 was at AGT, and 1 was at AGTR1. An SNP at CTP11B2 was seen in 5 separate pairs of SNPs. The authors postulate that sex-dependent and epistatic effects (ie, gene–gene interactions, which can often involve the suppression of one gene by another) could explain the well-known inconsistencies among previous studies of genetic effects on blood pressure (BP) and other phenotypes. Scurrah et al3 provide a Circos plot to represent the top 100 SNP–SNP interactions within and between each of the RAS genes studied.

These kinds of association findings, of SNP(s) and a phenotype, are all too common in molecular genetic studies. But what is the fundamental basis of such associations? To help foster thinking, I thought I would provide some mechanistic insights. These suggestions might prove helpful for further research by geneticists working in the cardiovascular and other fields.

The most obvious explanation is that a genetic lottery is at play. Individuals lucky enough to have inherited a net complement of genetic variants for low BP will avoid getting hypertension. At the level of an individual gene, a specific allele of 1 SNP or a combination of alleles of ≥2 SNPs might show an association with lower BP, while the contrasting allele(s) might be associated with higher BP. But this still does not explain the molecular mechanisms responsible.

What is it that mediates the effect of an SNP in or near a gene? An SNP can be functional or can simply be a marker for a functional variant with which it is in linkage disequilibrium (LD). If an SNP is not present in coding DNA and does not cause an amino acid change (as in the study by Scurrah et al3), one should consider the possibility that it might influence regulation of the gene. The DNA sequence of contrasting alleles of the SNP should be examined to see whether either spans a transcription factor–binding site (TFBS). If this is the case, it could be that a transcription factor might recognize only 1 allele of the SNP or it might bind more strongly to 1 allele than to the alternative allele. Using RegulomeDB,4 a database that annotates SNPs with known and predicted regulatory elements, including TFBSs, can reveal such potentially functional differences. These can be evaluated for their potential effects on binding affinity by comparing a variant with the accepted TFBS canonical sequence(s). Such potentially functional SNPs can then be mapped relative to known TFBSs (and RNA polymerase II–binding sites) using the WashU Genome Browser.5

Within a gene, an effect on BP or another phenotype may be more profound when individual SNPs cooperate. This can involve haplotype blocks (regions of LD between SNPs in a gene). Haplotype blocks can be ascertained through the HapMap database. SNPs can be screened using HaploReg, a tool for exploring annotations at haplotype blocks, such as candidate regulatory SNPs at disease-associated loci.6 Using LD information from the 1000 Genomes Project, linked SNPs can be visualized along with chromatin state and protein-binding annotation from the Roadmap Epigenomics and the Encyclopedia of DNA Elements (ENCODE) projects, sequence conservation across mammals, the effect of SNPs on regulatory motifs, and the effect of SNPs on expression from quantitative trait loci studies. The database FuncPred can also be used to reveal SNPs that may be functional. Thus, a functional SNP might act alone, with 1 allele perhaps contributing to an elevation in BP, or may act in concert with alleles of other BP-associated SNPs that form a cis-regulatory unit (high BP haplotype). Physically, aggregation of a transcription factor bound to its enhancer, combined with RNA polymerase II at the promoter to form a transcription complex, may be tighter for contrasting alleles of an SNP in the TFBS. This should lead to increased or diminished transcriptional activity. The overall effect on transcription could involve multiple transcription factors and SNPs, so causing the haplotype to have a stronger effect on, for example, BP than each individual transcription factor–SNP interaction.

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One of the intriguing concepts yet to be investigated in the cardiovascular field is the existence of long-range physical interactions across the genome. Such interactions represent gene regulatory networks devoted to a common theme, in this case BP. Interactions can span different chromosomes or can be confined to a gene neighborhood within the same chromatin domain of a chromosome. The existence of interaction(s) can point to involvement of gene(s) not previously implicated in, say, BP control. Long-range physical contact between genes involves the versatile DNA-binding protein CCCTC-binding factor zinc finger protein that binds to tens of thousands of sites across the genome by using different combinations of its 11 zinc finger domains to bind different DNA target sequences and proteins. Cis-regulatory elements are brought together into coregulated islands, and multiple islands are brought together into a functional neighborhood or archipelago by chromatin looping (see review by Montavon et al). These chromatin islands, referred to as topologically associating domains, can be detected through cross-linking experiments and are of the order of several hundred kilobase-pairs (kb), while archipelago connections can be on the order of 3 to 5 mega-base pairs (MB). Natural selection acts on the genome to maintain combinations of genes and their regulatory elements that are essential to fundamental biological processes. Chromatin contact points for a gene, and, thus, identification of coregulated genes in a gene neighborhood, can be investigated using the Epigenome Browser and Juicebox. This could reveal genes with previously unknown roles in, say, BP regulation. Coexpression of syntenic loci can be examined using GeneMania (which is the source of most of the data in the Gene Expression Omnibus). Tissue-specific expression patterns of neighboring genes can be ascertained from the Genotype Tissue Expression database (GTEx). In Drosophila, over 20% of the genome is composed of large domains of adjacent and similarly expressed genes, each having between 10 and 30 members. In humans, such genomic domains are likely to be much larger because of the larger size of the human genome. Connections between loci across multiple chromosomes can be determined. These kinds of studies might be regarded as a preliminary step toward discovery of the kind of physical connections and interactome that these 2 SNPs interact, one might wonder whether they cooperate in affecting one or other of these processes? The role of microRNAs and other noncoding RNAs in the genetic basis of hypertension is becoming an important avenue of research. Apart from allelic variants in microRNA target sites in the 3′-untranslated region of messenger RNAs, the possibility that genetic variants affecting expression of various microRNAs might contribute to the genetic basis of hypertension is worthy of investigation. Downregulation in human kidneys of a microRNA (miR-181a) that targets a site in the 3′-untranslated region of REN is associated with upregulation of renin expression, and this could contribute to hypertension by an effect involving an intrarenal RAS.

Four times as many non-coding as coding RNAs are transcribed across the human genome. Genetic variation in long non-coding RNAs in particular, but also small non-coding RNAs such as miRNAs, could contribute to differences in BP. Non-coding RNAs also affect the epigenome. Epigenetics is an emerging area in the hypertension field and could explain non-genetic (eg, environmental) effects.

In many genetic studies, as in the present one, association of SNPs with a trait can be sex dependent. The most obvious contributing factor to sex differences is sex hormones. It is well known that female sex hormones present prior to menopause provide protection against cardiovascular disease. If the authors had obtained data on menopausal status of the female participants, then this could have been addressed. Nevertheless, subgroup analysis of women by age, comparing those well short of the general age range of menopause with women likely to be postmenopausal might have been informative. This could have generated hypotheses worthy of testing, and so helping to elucidate the hormone mechanism responsible. An association of an SNP with BP in postmenopausal women likely to be postmenopausal might have been informative. This could have generated hypotheses worthy of testing, and so helping to elucidate the hormone mechanism responsible. An association of an SNP with BP in postmenopausal
women and in men, but not in premenopausal women, would provide data to foster other avenues of investigation.

The lack of consistency of findings for RAS genes over the years, and indeed of molecular genetic studies in the hypertension field and other complex traits generally, needs careful thought. Apart from the obvious usual suspects such as race, sex, cohort size (and, thus, power), gene coverage (number of SNPs tested), and differences in environmental factors between cohorts, age is an often-overlooked factor. Because hypertension is a risk factor for premature death, most often from myocardial infarction or stroke, subgroup analysis of younger and older subjects in a cohort should also be tested to determine whether an association with SBP or hypertension is seen in each subgroup. If a particular allele of a SNP is genuinely associated with SBP or hypertension, the association should be seen in both the younger and older subgroup. If not, one should consider the possibility of attrition of an SBP/hypertension risk allele because of increased mortality from a cardiovascular event earlier in life (middle age). Interestingly, such a phenomenon was initially reported for a variant in a RAS gene, namely the ACE insertion/deletion polymorphism. Survivor bias has the potential to invert a genetic association finding. In this example, depletion of subjects with the ACE D allele enriches I allele frequency in the group with hypertension to falsely suggest that the “I” allele is associated with hypertension when in fact there may be no association of either allele with hypertension.

To conclude, the comprehensive study of RAS gene polymorphisms and BP by Scurrah et al helps provide clarification of the role of these in genetic aspects of SBP variability. It now remains to elucidate the mechanisms involved. To this end, I trust that the suggestions I provide for further research might help in unraveling the fundamental molecular basis for the genetic contribution of SNPs in not just RAS genes, but in multiple other genes contributing to BP variation, and indeed a host of other phenotypes, as well as of hypertension and other pathological traits.

Disclosures

None.

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