Genetics of Blood Pressure – Still Hoping After All These Years

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Coming to terms with unfulfilled promises feels like the fate of those of us involved in the genetics of blood pressure. With the advent of human genome sequencing in 2000 it was expected that within 10 years, research would deliver clinically useful outcomes. Genetic discovery would not only accurately identify those at risk for high blood pressure, but also offer new means of prevention and treatment. How many successful grant funding applications were written with those very words? Yet, save for some terrific work in rare major Mendelian genetic causes of hypertension, common essential hypertension is still waiting for genetics to deliver something really useful.

Why so? The problems facing genetics (and not just for blood pressure) are essentially those of discovery and understanding.

Throughout, discovery has depended on the existence of differences in DNA sequence that can be used as map markers that might point to (and occasionally might be) DNA sequences that cause changes in physiology to affect blood pressure. The ability to detect these sequence variants is where technology has ruled. The advances here have been truly remarkable. Each step in the technological ladder has brought renewed hope that the problem will be cracked. Sadly, not yet.

Early genetic discovery was in rats using technically cumbersome restriction fragment length polymorphisms (RFLP) to demonstrate association between a polymorphism in the renin gene and blood pressure in the Dahl rat\textsuperscript{1}. Soon followed human studies of association built around candidate genes. By today’s standards these were very narrow analyses, often using few and often single markers. The association between the M235T polymorphism of the human angiotensinogen gene and blood pressure is a famous example\textsuperscript{2}. Unfortunately, if anything were to characterize the candidate gene era, it was inconsistency of results. Contradictory findings for the same markers frustrated readers and editors alike and the days of small (less than 1000 subjects) studies using limited markers were numbered.

Thankfully the era of whole genome analyses emerged at about this time with the availability of the genome sequence and the increased throughput technology that went with it.

Instead of focusing on a single gene, genomic studies scan all the chromosomes. Maps with 500,000 and 1 million markers soon became readily accessible. With about 13 million available in the human genome, the markers chosen were Single Nucleotide Polymorphisms
(SNPs). Suddenly the discovery of entirely novel genetic explanations outside the known candidate genes became a real possibility. The era of genome-wide association studies (GWAS) was upon us.

In a test of the new technology (as much as anything), the Wellcome Trust Case Control Consortium (WTCCC) applied GWAS technology to 7 common conditions: bipolar disorder, coronary artery disease, Crohn’s disease, hypertension, rheumatoid arthritis, type 1 diabetes, and type 2 diabetes(3). Using a common set of 3000 controls and about 2000 individuals with each of the 7 conditions, GWAS revealed associated loci with 6 of the conditions, but nothing of significance for blood pressure.

**Questions were being asked. Why was blood pressure resisting GWAS discovery? Was the blood pressure genetic architecture fundamentally different? Was the inherent variability in the blood pressure phenotype blurring the genetic focus?**

The response was to build bigger studies of 100,000 and more individuals. Inevitably these depended on compiling DNA and phenotypic data from large numbers of independent studies. This came with the price of heterogeneity, particularly in relation to blood pressure measurement protocols and methods, that did not improve phenotypic variability.

Notwithstanding, these mega-GWAS were able to identify around 80 chromosomal loci with effects on blood pressure(4). This was a major advance on WTCCC, but tinged with disappointment. Two problems stood out. First, the size of the effect of the individual loci was typically less than 1 mmHg and commonly less than 0.5 mmHg. Individually, these are clinically unmeasurable effects and of little use as predictive markers of blood pressure, let alone cardiovascular risk.

It was argued that it might be worth constructing a predictive test based on combining all the available markers – a so-called genetic risk score (GRS). However, the sum of the estimated effect of all the available markers could only account for less than 5% of blood pressure variance, while it had been expected that genetics should account for 40-50% of blood pressure variance. So, something was missing. Additionally, the number of individuals who carried sets of the existing markers became fewer and fewer as more markers were considered.

In any case, GRS with the best available markers in independent populations has not really improved risk prediction in any meaningful way beyond standard clinical risk evaluation(5).

One explanation for the unexpectedly poor yield of genetic discovery from GWAS might be analytical. A GWAS examines the probability of association with blood pressure 1 marker at a time. What if blood pressure effects weren’t obvious for individual loci, but were present when 2 or more markers (actually their adjacent causative loci) interacted to produce an effect greater than the sum of their parts? Such interaction is known as epistasis. Although there didn’t seem to be much evidence for epistasis in the GRS, these were limited tests for such effects. However, a more comprehensive genome-wide survey for epistasis is a statistical nightmare. Think of the daunting prospect of testing all the possible combinations of a million individual markers for association with blood pressure and the potential for false positives that would result.

There are other factors that might need be taken into account. One obvious one is sex. There are many reasons, from sex chromosomal loci to sex hormones themselves, why individual GWAS markers might only reveal their association with blood pressure in one sex. Lumping men and women together only dilutes such effects. Unfortunately, sex-specific analyses don’t figure prominently in contemporary GWAS.

Another phenomenon relevant to discovery is epigenetics. Here exogenous influences (broadly termed environmental) can influence the genetic expression. Chemical changes such as DNA methylation and histone modification(6) can be modulated by environmental exposure and affect gene expression, yet won’t be obvious when only the DNA sequence is measured. For example, the same GWAS marker might have different blood pressure implications depending on whether it is methylated or not. So, in 100,000 individuals in a large GWAS based on SNPs alone the association of a marker that depends on epigenetic modification could be missed.

Beyond factors (epistasis, sex and epigenetics) influencing the association of GWAS markers and blood pressure, it is important to recognise that the genome-wide net of 1 million SNP markers has many potential holes. Because individual SNPs are common and most often between genes (protein-coding genes make up about 2% of the genome) they are not suited to detecting infrequent or rare variants that might influence the protein products of gene expression.

Early studies suggested that although infrequent, DNA variants in the coding sequences of genes (the exomes) might be present in around 1 in 1000 or so individuals and impart an effect on blood pressure of potentially measurable significance – around 5 mmHg(7).

Hence the next logical step in discovery was the genome-wide study of exomes – so-called exomics. Ideally this would involve screening the actual DNA
sequences of exomes in large numbers of individuals. However, with studies of several 100,000 people, the most recent studies have used chip technology based on known markers that are of low frequency (1-5%) or rare (<1%). Two large recent studies used the same Exome Chip and both resulted in the discovery of coding sequence markers associated with blood pressure(8,9). There was some overlap in the findings, but differences also. Interestingly the most consistent marker associations were those for diastolic blood pressure. Moreover, rare variants were hard to find and most of the markers were associated with an estimated blood pressure effects of less than 0.5 mmHg. So, still there remains a gaping hole in the molecular genetic answers to the heritability of blood pressure, with less than 10% of variance explained.

This is all very frustrating. Despite the enormous resources spent to date, the returns have been small. Indeed, the inverse relation between the size of the study and the magnitude of the blood pressure effects discovered must leave funding bodies wondering whether or not present strategies for genetic discovery are becoming cases of diminishing returns.

For those of us from a more physiological, less molecular era, the more important questions relate to understanding exactly what the markers associated with blood pressure actually mean and the mechanisms by which causal variants might operate.

There are 2 basic approaches to understanding here. One is to try and deduce what the DNA sequence variants might imply downstream. The other is to place the variants in model systems and organisms and study their real-life effects. In reality, both are required and its sensible to begin by identifying the presumed molecular culprits before investing in functional experiments.

It's important to appreciate that the markers used for discovery are not necessarily (or even often) the DNA variants that influence blood pressure. However, we can be assured that they are in close proximity on the chromosome.

Around a particular marker there might be many (sometimes thousands) of potentially important variants. Finding the right one is predicated on understanding the operation of human genome, particularly those parts outside the protein-coding regions. These intergenic regions comprise sequences that code for things such as non-coding RNA that manage the sophisticated coordination of gene expression in particular cells at certain times of life(10). They also influence the epigenetic interactions between environment and the genome and likely a host of other factors, yet to be defined. Our relative ignorance of these factors limits our ability to decide which of a list of variants around a marker might be the target for further study. Yet these intergenic regions are precisely where the majority of the GWAS markers associated with blood pressure reside.

We are at something of a crossroad at the moment. We have accumulated masses of data and potential markers from the GWAS and exomic studies so far. And we are likely to accumulate much more with Next Generation Sequencing technologies. Do we continue down these paths with larger and larger studies with more subjects and more markers? How likely is it really that by continuing down this path that a genetic variant of major effect on blood pressure will suddenly reveal itself?

More effort should be devoted to working with the information we have to date. It's time to curate rather than just catalogue the collection. Investigate the markers that associate consistently across the various studies, identify the causative variants and determine by what mechanisms they influence blood pressure.

In the end, how will all this effort for the genetics of blood pressure be judged? Here the family doctor might have the appropriate threshold question. Will genetic discovery and understanding add anything significant to my ability to predict cardiovascular risk beyond simple blood pressure measurement and clinical assessment, and will there be changes to my ability to reduce blood pressure beyond current effective treatments?

**Only time will tell, but we are still hoping.**

**REFERENCES:**


3. Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007, 447:661-678.


- Stephen Harrap and Fadi Charchar