REVIEW

Cardiovascular Therapeutics

The Genetics of Blood Pressure and Hypertension: The Role of Rare Variation

Peter A. Doris

Center for Human Genetics, Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, Houston, Texas, USA

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Correspondence
Peter A. Doris, Brown Foundation Institute of Molecular Medicine, 1820 Pressler, Suite 530F, Houston, TX 77030, USA.
Tel.: +1-713-500-2414;
Fax: +1-713-500-2447;
E-mail: Peter.A.Doris@uth.tmc.edu

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SUMMARY

The role of heredity in influencing blood pressure and risk of hypertension is well recognized. However, progress in identifying specific genetic variation that contributes to heritability is very limited. This is in spite of completion of the human genome sequence, the development of extraordinary amounts of information about genome sequence variation and the investigation of blood pressure inheritance in linkage analysis, candidate gene studies and, most recently genome-wide association studies. This paper considers the progress of this research and the obstacles that have been encountered. This work has made clear that the genetic architecture of blood pressure regulation in the population is not likely to be shaped by commonly occurring genetic variation in a discrete set of blood pressure-influencing genes. Rather heritability may be accounted for by rare variation that has its biggest impact within pedigrees rather than on the population at large. Rare variants in a wide range of genes are likely to be the focus of high blood pressure genetics for the next several years and the emerging strategies that can be applied to uncover this genetic variation and the problems that must confronted are considered.

Introduction

Blood pressure is a heritable trait with estimates of heritability indicating that 30–70% of the trait variance is attributable to genetic variation [1–8]. The progress of genetic investigation to identify the genes that contain this variation and thereby affect blood pressure regulation and influence the risk of hypertension has reached a new plateau with the publication in the last year of several large scale population genetic studies that have surveyed very large numbers of stable genetic markers in an attempt to associate individual markers with blood pressure and hypertension [9,10]. The application of such genome-wide association studies (GWAS) in populations is an important technical achievement requiring not only large and well-characterized populations, but the capacity to accurately type very large numbers (hundreds of thousands) of single nucleotide polymorphisms (SNP's) in these individuals. This work has accomplished some progress, however, much of this progress has been to test, and largely reject, the hypothesis that a substantial amount of the heritability of blood pressure is associated with a discrete number of relatively common genetic variants. New insight has been achieved, but perplexing issues remain. In this respect, the current situation is similar to the outcome of initial mapping efforts that employed linkage analysis in pedigrees that were reported several years ago [2,11–19]. It is timely to consider how research into the genetic basis of high blood pressure has reached its present point, to consider the unexplained role of heritable factors that seems to remain and to reflect on the prospects for and obstacles to further progress.

The Genome Sequence and its Variation Provide the Substrate for Blood Pressure Genetic Studies

The human genome project was founded in 1990, a rough draft sequence was announced in 2000 and a completed sequence in 2004. This large-scale effort has been marked with extraordinary technical accomplishments driven by the great potential value expected to emerge from knowledge of the detailed sequence of the human genome. The finished genome sequence has been but one of many resources the genome project has enabled. As knowledge of the genome sequence expanded, increased knowledge of sequence variation was also obtained. Dense and detailed knowledge of human genome sequence variation has been a key resource in leveraging mapping efforts to identify the genetic variation that contributes to many common diseases, including hypertension. Recognizing the value of knowledge of sequence variation, the HapMap project was initiated as an effort to sample the common genetic variation in diverse global populations [20]. Remarkable advances in the efficiency of whole genome sequencing has

lead to the 1,000 Genomes Project which is currently under way, collecting DNA samples from a very diverse array of human subpopulations representing each major continent as well as several admixed populations [21]. This project will provide much more information about human genetic diversity and population-specific genetic variation.

The first systematic approach to examine whether the whether genetic markers could be found that were consistently linked to blood pressure levels or the presence of hypertension was performed in mapping studies of populations comprising related individuals. These studies were propelled forward by the excitement that the highly successful application of this approach to single-gene diseases had generated. In contrast, the outcome of linkage mapping studies in complex genetic disease was marked by rather modest progress [22,23]. The studies reported were limited by relatively low analytical power, a lack of positional resolution, identification of a limited number of linked genomic regions, poor replication of these findings within comparable populations and across populations of different ethnic composition, and modest effects associated with the linked markers. A better way to approach the mapping problem was needed that combined new technical breakthroughs that made possible the use of much larger numbers of SNP markers in mapping with a theoretical framework to account for the role of population genetic variation in prevalence of high blood pressure.

Common Variation in Common Disease

In 1996, Risch and Merikangas proposed a new approach to the problem of identifying genetic variation contributing to complex genetic disease susceptibility [24]. This new approach was appealing not only because one was clearly needed for the field to advance, but also because it articulated a rationale that, by coupling recent human demographic history to an evolutionary framework, offered the opportunity to make discoveries of potentially broad impact. This framework proposed that, since humans are a relatively new species that has dispersed across the planet only in the last 40 millennia or so, and since the propensity for common diseases, such as high blood pressure, seems to exist in all human populations, then the component of this propensity that is explained by genetic variation may have existed in ancestral humans prior to the global diaspora. In order for such genetic variation to be preserved so widely among diverse human populations, the frequency of such variation must be relatively high. Thus, with information on the extent and distribution of genetic variation beginning to accumulate, the task of determining which common variation might play a role in disease risk was starting to come within reach.

Implementation of the experimental approach to test the common disease:common variant (CD:CV) hypothesis involves population-wide genotyping of very large numbers of common SNP variants to determine which variants shows significant association with blood pressure or hypertension. Such large-scale genotyping provides a means to directly identify the causal variants (or reveal them through linkage disequilibrium as likely contained within haplotype blocks in adjacent regions of the genome). This approach is not without its obstacles. One is the sheer scale of the endeavor: a survey of 500,000 SNP's per individual in a population of 10,000 individuals requires 5 billion individual genotype tests—a significant analytical challenge. Improvement in genotyping technology has allowed generally robust and reliable genotyping methods [25,26]. It also creates a multiple testing problem since each SNP is tested individually for association with the trait(s) of interest. The multiple testing problem is accommodated by adjustment of *P*-values, requiring very low *P*-values (typical marker sets require around $<1 \times 10^{-8}$) to reject the null hypothesis that any given SNP is not associated with the trait. One minor risk of this approach is the presumption that all variation needed to accomplish this type of study can be sampled directly (or indirectly because it lies in strong linkage disequilibrium nearby a SNP that has been sampled) so that none, or only a small fraction, of any detectable causative variation would be missed with the large number of SNP's sampled.

The overall performance of the technical elements of such large-scale GWAS has been well substantiated by results generated when this type of investigation was applied to several common diseases and by the capacity of GWAS studies to replicate findings obtained in independent samples. For example, replicated findings for specific markers associated with disease risk have been identified in diseases such as prostate cancer [27–29], type 1 diabetes [30–33], and type 2 diabetes [34,35]. A full inventory of published GWAS studies is maintained by the NIH Human Genome Research Institute (http://www.genome.gov/gwastudies/). Success, as usual in complex disease genetics, is a label that has to be carefully defined: although associations between SNP markers and disease traits have been found and replicated, this does not imply that the marker is the causal variant, or even that the causal variation is in the gene containing or closest to the associated variant [36]. Progress in uncovering such causal variants and proving their functionality remains at an early stage.

Blood Pressure and Hypertension GWAS Emerges, but Produces Limited Insight

Thus, with a mature and robust analytical approach, initial efforts to apply GWAS to blood pressure and hypertension were reported in 2007. As might be expected, these initial studies were somewhat exploratory in nature, providing an important opportunity to outline the parameters (population size, quantitative versus dichotomous phenotypes, marker numbers, control populations, statistical analysis) needed to accomplish discovery. The first such published study was performed by the Wellcome Trust Case Control Consortium (WTCCC). This ambitious project sampled a northern European population using GWAS to investigate seven common diseases [37]. The experimental design used 2,000 cases for each of the diseases examined and 3,000 combined controls coming from two distinct cohorts, SNP's were typed using the Affymetrix 500K Mapping Set. Although successful for some disease traits, the WTCCC project failed to find any markers with alleles that occurred with significantly more frequency in hypertensive subjects than in controls. This was a disappointing initial outcome. However, the contrast between the disease-affected and control populations was substantially diluted. This dilution resulted from the fact that the control population was not screened to remove

individuals with hypertension. As a common disease, there was presumably a relatively high frequency of hypertension among the controls that reduced the power of the contrast between the control and affected samples. The outcome of the primary hypothesis that SNP's could be identified that were significantly associated with hypertension was not supported by this initial effort and consequently the genetic architecture of heritable susceptibility to hypertension was left clouded.

Efforts to clarify the picture have included follow up studies that have focused on SNP's identified in the WTCCC study that came closest to statistical significance. Studies in the US-based Family Blood Pressure Program cohort examined these SNP's in populations of predominant European, African Americans and US Hispanic ancestry [38]. The FBPP, like the WTCCC, is a consortium of several distinct study populations and over 11,000 individuals participated in the study. The study design was not GWAS, but rather explicit replication test of the 6 SNP's with highest p values obtained in the WTCCC. Only one of the 6 SNP's tested was found to be associated with blood pressure levels in the FBPP study. It is notable that another distinction between the FBPP study design and the WTCCC design is that the FBPP examined the relationship between the SNP's and the continuous trait of blood pressure as well as the dichotomous trait of hypertension while the WTCCC was able only to examine hypertension status. The use of continuous traits (systolic and diastolic BP) increases study power compared to the dichotomous trait of hypertension examined in the WTCCC study. In addition, the FBPP study population comprised related individuals and so was able to use transmission disequilibrium tests to examine the relationship between inheritance of SNP alleles and traits. The findings in this study are both impressive and perplexing. A single SNP (rs1937506) was shown to be associated with blood pressure in European Americans for which the effect size of a single copy of the G allele was estimated at −25 mm Hg. This is an unexpectedly large effect. This marker does not tag a gene or a genomic region that has been identified previously through alternative investigational approaches as containing variation influencing blood pressure. This is rather surprising, though possible explanations can be conceived. Remarkably, in Hispanic Americans the same allele contributed to an *increase* in blood pressure of +28 mmHg, a very large opposite effect. While this could be explained by different background allele frequencies in this population or by the presence of some other variation in linkage disequilibrium with rs1937596, the size and opposite effect of this variant remains insufficiently explained. In African Americans the same allele had no effect on blood pressure. In spite of its large effects on blood pressure in European and Hispanic Americans, the SNP was not found to be significantly associated with the affected status (hypertensive vs. normotensive). Adding to the perplexity, the location of the SNP in a region of the genome lacking nearby genes precludes any simple relationship between the SNP (or adjacent sequence variation) and known functional pathways affecting blood pressure regulation. An investigation of the same six variants from the WTCCC study in an Asian population produced a mixed result [39]. Two SNP's (rs6997709 and rs7961152) were associated with systolic and diastolic blood pressure, respectively, while the latter SNP (rs7961152) was also found to be associated with hypertension status. Thus, surprisingly disparate results are observed for both SNP's and for ethnicities in this effort to replicate and confirm the WTCCC findings.

Additional GWAS studies of blood pressure and hypertension have been reported. These studies have been performed using a range of populations including genetically isolated European American Amish [40], African Americans [41], Europeans [42], and Asians [43]. Table 1 summarizes the populations studied and the main findings. The studies have generated interesting and potentially important positive findings. However, the results obtained are varied with respect to the loci and markers uncovered. This outcome is not inconsistent with the CD:CV hypothesis. However, the findings do not suggest that the high frequency of hypertension in the populations studied results from the existence of a *limited number* of shared hypertension loci. This raises the possibility of extensive heterogeneity in the genetic loci contributing to hypertension both within and among populations. The emerging picture also clearly indicates that those loci that are being detected in GWAS are associated with very minor effects on blood pressure. Another emerging feature is that while some blood pressure-associated SNP's are clearly located within or nearby genes, other are located in gene deserts. For the latter variants, this implies a difficult path forward in the anticipated functional validation of these variants.

GWAS Moves to Meta-Analysis

The value of increasing analytical power in GWAS studies by increasing the size of the study population has not been overlooked and has resulted in concurrent publication of two large studies in which population sizes have been expanded dramatically. In both cases, this was achieved by combining samples from a number of medium- to large-scale epidemiological studies. The first, known as the Global BPgen study examined SNP association with blood pressure in 34,433 subjects of European ancestry with follow-up studies in over 80,000 subjects of European and South Asian ancestry [9]. The CHARGE consortium performed its discovery study using genotype data from nearly 30,000 North American subjects and used the Global BPgen discovery population for follow-up of its most significant associations [10]. The results of these studies are summarized in Table 2. Together the two studies have served to solidify the emerging picture from GWAS studies: given sufficient power, these studies can find reliable blood pressure associations; the blood pressure associations discovered are generally also hypertension status associations; the associations can contain substantial overlap across different populations; when these associations arise from markers within or nearby genes, these genes are largely not in prominent pathways known to have major effects on blood pressure regulation that are targeted by mainline antihypertensive pharmacotherapy; and the blood pressure and hypertension risk effects associated with these discoveries are consistently small.

While the accomplishments made by GWAS in hypertension and blood pressure are important, a substantial fraction of their importance arises from what they have failed to reveal, rather than what they uncovered. Most notable is their failure to generate observations that are able to account for the high estimated

Table 1 Initial GWAS and follow-up studies of blood pressure and hypertension status (ND = not done)

Authors	PubMed ID	Population	n	Trait	SNP's typed	SNP/Gene associated	Metaanalysis	max GWAS *P* value	Effect size (mm Hg)	Replication	Replic result
Wang et al	19114657	Amish	542	BP	80K	rs4977950	Y	9.1×10^{-8}	3.3	Y	Confirmed
						STK39 (cluster of SNPs)	Y	8.9×10^{-6}			
Cunnington et al	20003416	British caucasians	1372	BP	3	STK39	N	NS	None	ND	
Org et al	19304780	Europeans	1017	Hyp	1017	CDH13	Y	5.3×10^{-8}	1.6	Y	Marginal
Cho et al	19396169	Asians	8842	BP	352K	rs17249754/ATB2B1	N	9.1×10^{-7}	1.3	Y	Confirmed
						rs715987	N	4.5×10^{-6}	1.6	Y	Not confirmed
Adeyemo et al	19609347	African Americans	1017	BP/Hyp	800K	rs5743185/PMS1		2.1×10^{-11}	~5–6	ND	
						rs16877320		3.4×10^{-9}		Y	Not confirmed
						rs11160059/SLC24A4		1.5×10^{-8}		Y	Not confirmed
						rs17365948/YWH AZ		1.6×10^{-8}		Y	Not confirmed
						rs12279202/IPO7		4.8×10^{-8}		ND	
						rs3751664/CACN A1H		6.7×10^{-8}		ND	

heritability of blood pressure. As the field of hypertension population genetics has moved forward, over the course of more than a decade, from relatively modest study designs of linkage-based mapping in populations of related individuals to very large scale populations of unrelated individuals, much of what might have emerged from these studies has not emerged: no gene variation that contributes substantial effects on blood pressure has been found as a result of them; concurrence between linkage mapping studies and GWAS has been negligible; genetic information that would contribute in a clinically meaningful sense to the prediction of disease risk in individuals has not emerged; no new (or old) targets of pharmacotherapeutic development have been uncovered; and no insight into how genetics might lead to personalization of hypertension therapy (pharmacological or nonpharmacological) has been obtained. In a disease where adherence to pharmacotherapy is low and existing therapeutics lack specificity for the underlying pathogenetic mechanism, perhaps contributing to high rates of undesirable side effects, progress identifying either rational new drug targets or links between genes and existing drug targets would be valuable. In addition, no cohesive picture of the functional pathways through which genetic variation might synergize to enhance risk of hypertension has come to light. Only one study has attempted to infer pathway pathogenesis pathways based on GWAS findings and has done so based on GWAS hits that are either unreplicated or that failed to replicate when tested [41].

The Heritability Gap

The presumed role of common variation in blood pressure regulation has encouraged large investments in GWAS. The exciting sense of opportunity has synergized with rapid technological advances in genotyping to make the performance of these studies irresistibly compelling. Did these forces dampen scrutiny and critique of the presumptions that underlie GWAS of blood pressure? One central issue arises from well-accepted evidence that blood pressure and hypertension status show relatively high heritability. If 30–70% of blood pressure variance is correctly attributable to genetic variation, then clearly GWAS studies have failed to make more than a trivial dent in even the lowest estimates of heritability and there remains a large amount of genetic discovery left to make. The alternative is that the methods used to estimate human heritability in these traits have overestimated genetic factors and underestimated other influences (shared environment, diet, age, exercise, BMI). Estimation of heritability is limited because the other variables involved (environmental and phenotypic variation), are difficult to measure precisely. Indeed, in the case of blood pressure, the phenotype is a highly dynamic variable with substantial sampling issues. Measurement of heritability among related individuals inevitably results in increased sharing of environment, which may have important effects both in the time frame of the estimate (individuals sharing the same current environment) and even greater effects in the long term (individuals that have shared a similar environment, potentially from conception). However, even if the heritability of blood pressure has been generally overestimated, the extent of any such overestimation

Table 2 Summarized Findings in Two Large Scale GWAS studies of blood pressure and hypertension status (modified from www.genome.gov/gwastudies). Replicated genes are shown in bold

First Author	PubMed ID	Trait/Disease	Initial Sample Size	Replication Sample Size	Region	Reported Gene(s)	Strongest SNP-Risk Allele	Risk Allele Frequency in Controls	*P*-value	Odds ratio or beta-coefficient and [95% CI]
Newton-Cheh	19430483	Diastolic BP	34,433	100,347 Europeans,	15q24.1	CYP1A1, CYP1A2, **CSK**, LMAN1L, CPLX3, ARID3B	rs1378942-C	0.36	1×10^{-23}	0.43 [0.35–0.51] mm Hg increase
				12,889 Indian Asian	4q21.21	FGF5, PRDM8, c4orf22	rs16998073-T	0.21	1×10^{-21}	0.5 [0.40–0.60] mm Hg increase
					12q24.12	ATXN2, **SH2B3**	rs653178-T	0.53	3×10^{-18}	0.46 [0.36–0.56] mm Hg decrease
					10q21.2	c10orf107, TMEM26, RTKN2, RHOBTB1, ARID5B	rs1530440-T	0.19	1×10^{-9}	0.39 [0.27–0.51] mm Hg decrease
					17q21.32	ZNF652, PHB	rs16948048-G	0.39	5×10^{-9}	0.31 [0.21–0.41] mm Hg increase
					3q26.2	**MDS1**	rs1918974-T	0.54	8×10^{-8}	0.27 [0.17–0.37] mm Hg decrease
		Systolic BP	34,433	100,347 Europeans,	10q24.32	**CYP17A1**, AS3MT, CNNM2, NT5C2	rs11191548-T	0.91	7×10^{-24}	1.16 [0.92–1.40] mm Hg increase
				12,889 Indian Asian	1p36.22	MTHFR, NPPA, CLCN6, NPPB, AGTRAP	rs17367504-G	0.14	2×10^{-13}	0.85 [0.63–1.07] mm Hg decrease
					17q21.31	PLCD3, ACBD4, HEXIM1, HEXIM2	rs12946454-T	0.28	1×10^{-8}	0.57 [0.37–0.77] mm Hg increase
Levy	19430479	Diastolic BP	29,136	34,433	12q24.12	**SH2B3**	rs3184504-T	0.48	3×10^{-14}	0.48 [0.36–0.60] mm Hg increase
					15q24.1	**CSK**, ULK3	rs6495122-A	0.42	2×10^{-10}	0.4 [0.28–0.52] mm Hg increase
					12q21.33	ATP2B1	rs2681472-A	0.83	1×10^{-9}	0.5 [0.34–0.66] mm Hg increase
					3p22.1	ULK4	rs9815354-A	0.17	3×10^{-9}	0.49 [0.33–0.65] mm Hg increase
					10p12.33	CACNB2	rs11014166-A	0.66	1×10^{-8}	0.37 [0.25–0.49] mm Hg increase
					12q24.21	TBX3, TBX5	rs2384550-A	0.35	4×10^{-8}	0.35 [0.23–0.47] mm Hg decrease
					11p15.1	PLEKHA7	rs11024074-T	0.72	1×10^{-6}	0.33 [0.19–0.47] mm Hg decrease
		Systolic BP	29,136	34,433	12q21.33	ATP2B1	rs2681492-T	0.8	4×10^{-11}	0.85 [0.60–1.10] mm Hg increase
					10q24.32	**CYP17A1**	rs1004467-A	0.9	1×10^{-10}	1.05 [0.74–1.36] mm Hg increase
					11p15.1	PLEKHA7	rs381815-T	0.26	2×10^{-9}	0.65 [0.43–0.87] mm Hg increase
					12q24.12	**SH2B3**	rs3184504-T	0.48	5×10^{-9}	0.58 [0.38–0.78] mm Hg increase
					3q26.2	**MDS1**	rs448378-A	0.52	1×10^{-7}	0.51 [0.31–0.71] mm Hg decrease
					10p12.33	CACNB2	rs11014166-A	0.66	7×10^{-7}	0.5 [0.30–0.70] mm Hg increase
					1p36.22	CASZ1	rs12046278-T	0.64	5×10^{-6}	0.53 [0.29–0.77] mm Hg decrease
		Hypertension	29,136	34,433	12q21.33	ATP2B1	rs2681472-A	0.83	2×10^{-11}	0.15 [0.11–0.19] increase in log odds
					10p12.33	CACNB2	rs11014166-A	0.66	6×10^{-8}	0.09 [0.05–0.13] increase in log odds
					20q13.32	ZNF831, EDN3	rs16982520-A	0.88	2×10^{-7}	0.13 [0.09–0.17] decrease in log odds
					8p23.1	MSRA	rs11775334-A	0.32	4×10^{-6}	0.08 [0.04–0.12] increase in log odds

seems unlikely to account for all of the missing heritability that remains to be accounted for after GWAS.

Continued progress of genetic studies of blood pressure and hypertension requires some framework to account for the failure of common variants affecting these traits to be identified by GWAS. One potential explanation is that GWAS has failed to uncover underlying variants because it was designed to sample common variants (typically those with allele frequency greater than 2–5%). By exclusion, this suggests that rare variants may play a greater role in heritability than envisioned in the CD:CV hypothesis. The efficiency of GWAS to detect trait effects arising from rare variants is limited. Individual rare variants make their phenotypic impact in families segregating these variants and are diluted in cross-sectional population analysis. Within families, rare variants may have much greater effect sizes than those found for common variants and may account for the greater heritability. For common traits like hypertension, this implies that many rare variants might exist within a population that can influence the trait, a situation that poses a new set of problems for their successful detection and verification.

Rare Variants Step Forward

Pioneering work by Richard Lifton and colleagues has begun to outline the contours of the role that rare variants may play in hypertension. Over the course of several years, this group has made remarkable progress in mapping genes and mutations responsible for rare Mendelian forms of hypertension and their converse counterparts, mutations that lead to syndromes of reduced blood pressure. These discoveries have been in harmony with, and indeed have further confirmed, an important theoretic construct in the systems analysis of blood pressure, namely that the only way to affect blood pressure in the long term is to alter renal sodium handling [44,45]. Each of the genes reported to influence blood pressure has been shown to function in an important renal pathway acting on sodium reabsorption or its regulation. While it is clear that the Mendelian mutations that Lifton and colleagues have uncovered contribute insignificantly to population hypertension risk, the possibility exists that genes whose effect on blood pressure regulation is sufficiently large to allow Mendelian traits to emerge may also contain other variation affecting the same traits.

Data is now in hand that sheds some light on this question. SLC12A1 and KCNJ1 are genes contributing to Barrter's syndrome, while mutation in SLC12A3 causes the less severe Gitelman's syndrome, both are rare Mendelian salt-wasting diseases associated with low blood pressure. The known disease-causing mutations in these genes occur in the homozygous autosomal recessive state with frequencies of ~1 per million and per 40,000 for Barrter's and Gitelman's diseases, respectively. Resequencing these three target genes in the Framingham Heart Study population (1,985 subjects and 1,140 relatives) has identified novel variation in these genes and defined the frequency of such variation in this population [46]. The resequencing effort identified 46 synonymous substitutions, 89 missense substitutions, one nonsense mutations and two frameshift mutations. Twenty-three subjects contained a total of 10 distinct, previously known and biochemically proven, loss of function mutations. Mutations distinct from these 10 known mutations were assessed as potentially deleterious when they alter amino acids that are highly conserved. The final yield of potentially deleterious variation was 30 distinct mutations present in 49 subjects. A rather unorthodox analytical approach was then applied. The carriers of potentially deleterious mutations in all three genes were treated as a group and analyzed to determine whether blood pressure levels differed in this group compared to the Framingham Heart Study cohort. Systolic blood pressure was found to be lower (by 5.7 mmHg at age 40 and by 9.0 mmHg at age 60) in the mutation-bearing group. Analysis was also carried out within families comparing sibs that carried a mutant allele with sibs not carrying such an allele. Lower blood pressure was also observed in the mutant-allele carriers. Furthermore, incidence of hypertension was lower in the mutation-carrying group than in the Framingham cohort.

These studies reveal that 1 in 64 members of the Framingham cohort may carry a mutant allele that affects blood pressure, when the mutation is selected for an effect on a conserved amino acid and when pooled across three genes. In aggregate, these mutations appear to reduce the risk of hypertension by 60% at age 60. It is unlikely that any of these variations would have come to light in a GWAS study of common variation since the frequency of each individual variant is lower than the threshold for classification as common. Thus, these studies have begun to clarify the possibility that the risk of hypertension in at least some fraction of the population can be strongly affected by functional rare variation. Given the possible deleterious effects of the homozygous state of such mutations on subject health, it might be expected that purifying selection would eliminate them. But this may not be the case. In an interesting modeling study to address the possible role of rare variants in common disease, Pritchard has concluded that it is feasible that common disease traits might result from rare variants [47]. This modeling suggests that loci harboring rare variants affecting common diseases could be characterized by relatively high mutation rates. This would lead to extensive disease allele heterogeneity, sufficient to account for disease in the presence of weak purifying selection. These predictions, and similar ones by Eyre-Walker [48], appear to harmonize quite well with observations in the Framingham cohort. Thus, it is reasonable to predict that other loci affecting blood pressure in the population will also be loci containing genes influencing renal sodium reabsorption, will also be subject to relatively high mutation rates and will therefore incorporate extensive allelic diversity of rare alleles among the disease-associated alleles.

It is worthwhile to consider that the Framingham study identified 92 missense, frameshif,t and nonsense mutations in the three target genes. Conclusions about the effect of rare mutations on blood pressure and hypertension risk were limited to just those 30 mutations either previously shown to cause Gitelman's or Barrter's syndromes (10 SNP's) or those altering highly conserved amino acids (20 SNP's). And these conclusions were reached in a novel, unorthodox manner. The typical hypothesis to test association between sequence variation and disease tests the relationship to a single variant. In their studies of Barrter's

and Gitelman's syndrome gene variation the relevance to blood pressure and hypertension was revealed through a hypothesis that excluded amino acid-changing variants that affected amino acids with lower rates of conservation and pooled variation so that not only was more than one variant tested concurrently, but variants in more than one functional gene were also concurrently tested.

The work on rare variants in the Framingham cohort highlights two important potential difficulties that will have to be overcome to clarify fully the role of rare variants in influencing blood pressure and hypertension. The first issue that will have to be confronted is that of the appropriate formulation of hypotheses to test rare variation. The most parsimonious approach is to test individual variants for trait effects. However, this would certainly require populations much larger than the Framingham cohort because these variants will be sufficiently rare that detection of their effects will require the power afforded by a sufficient number of carriers [49]. If allelic heterogeneity is a feature of most genes contributing to rare variation, as Pritchard's modeling and the Framingham investigation suggest, then more often than not many variants in each gene will need to be tested individually. The alternative approach of testing multiple variants concurrently relies on assumptions that are only beginning to be tested. For example, that all nonsynonymous mutations affecting amino acids that are highly conserved are deleterious to protein function. Some variants may yield gain-of-function mutations and consequently, may have opposing effects on trait values or status. Although gain of function mutation is undoubtedly less frequent, the proposal to ignore them introduces some hazard [50]. Thus, pooling variants that have untested and possibly divergent trait effects may obscure real effects associated with individual variants. Allelic heterogeneity poses a practical problem whose solution is not yet clear. Perhaps only increasing sample size will prove adequate, though upper bounds on required sample sizes may be very large indeed if the effect associated with the variant is small [49]. In the Framingham study, there are many variants including those creating nonsynonymous amino acid changes that, as a result of the rationale formulated for testing effects of variants, are not tested. Is it always reasonable to exclude such variants from a pooled analysis, or is it reasonable only when newly discovered variation can be compared against a diverse pool of biochemically and functionally characterized disease alleles to ensure similar properties are shared? What effect might variants affecting level of gene expression contribute to phenotypes? If pooling is to be used, is it possible to concurrently test variants that affect both expression level and protein composition? The acid test of functional genetic variation has always been to express the variant protein and demonstrate altered functional properties consonant with the trait effect. The sheer number of potentially relevant variants will make this a daunting task. Intermediate steps that extend simple properties of amino acids changes as affecting conserved or nonconserved [46], or presume all nonsynonymous variation to be deleterious [50] or that encompass prediction of altered biochemical properties of substituted amino acids on broad aspects of protein function may provide a workable substitute for the testing of each individual variant, but such indirect approaches are uncertain and venture into unfamiliar territory.

Rarely A Way Forward

These insights begin to outline a strategy to detect blood pressure and hypertension trait variation. Such a strategy would target genes known to affect or effect renal sodium reabsorption, would examine relatively large and well-characterized populations, would employ extensive resequencing of the target genes, and would characterize the resulting variation with respect to the possibility and predictability of functional effects of mutation. Since some of the functional variation might arise from sequence variation in noncoding regions that acts to alter transcription, protein abundance and activity, extension of this strategy beyond the coding regions of genes is likely to be necessary. Compound heterozygosity must also be considered since allelic heterogeneity may result in large trait effects arising from heterozygosity for two distinct nonwild type alleles [51]. In the field of blood pressure and hypertension, the range of genes that might be targets of such studies could be very large, reflecting the role of diverse regulatory systems (autonomic, endocrine, autocrine, vascular, endothelial, and renal epithelial) capable of influencing blood pressure. While existing studies have emphasized the role played by renal sodium transport genes and their local regulatory pathways in affecting blood pressure [52,53], it is conceivable that other genes, including genes not expressed in the kidney, might act indirectly on renal sodium reabsorption.

The selection of such functional candidate genes to include those that may be subject to more frequent mutation and hence more likely to contribute to high diversity of deleterious alleles awaits emerging whole genome sequencing studies of genetic variation in multigeneration pedigrees that may allow prioritization among potential candidate genes. One such study has been published and has clarified and confirmed prior estimates of the genome-wide de novo mutation rate by directly comparing the genome sequences of parents and offspring [54]. These studies also support prior expectations that higher mutation rate in the male germ line contributes asymmetrically to overall mutation and confirm the expected high rate of mutation affecting CpG sequence. The recent publication of small scale whole genome sequencing studies suggests the imminent arrival of sufficient cross-generational whole genome sequence data to establish whether local differences in mutation rate can be identified and whether this might be a useful tool to refine the candidate gene list for the identification of rare deleterious mutations by resequencing in large populations.

Concluding Thoughts

The path traveled as human geneticists have struggled to shed light on how risk of hypertension is transmitted in populations has been frustrating and challenging. It is without doubt a difficult problem. The main findings so far are that, in sharp contrast with Mendelian traits, there are no simple answers: there are no genes with alleles that account for a large fraction of the total population variation in blood pressure and hypertension risk. Common variants can be identified that affect blood pressure, but their contribution is much less than anticpated. While these findings are generally negative, they have an important significance in shaping the

battlefield: either heritability estimates are dramatically incorrect or, more likely, these traits are affected by alleles that are quite heterogeneous and uncommon. The loci that contain genes with alleles affecting blood pressure may also be heterogeneous when considering the population broadly. Indeed, this seems likely to be the case: otherwise linkage analysis should have been a more productive approach to identifying such variation. The best explanation for the lack of success of linkage studies is that their power is eroded by extensive heterogeneity of blood pressure loci in the population.

Platt and Pickering were antagonists in a controversy that engaged the hypertension research community for many years during the 20th Century [55]. The chief element of their dissent was the belief on one side that the heritability of hypertension was simple and Mendelian, while on the other, that the blood pressure was a continuous trait and therefore undeniably the result of polygenic inheritance. Clarity was obstructed by the difficulty in precise assessment of this dynamic and intrinsically variable trait and the absence of genetic tools that might provide definitive answers. The current epoch of genome-wide sequencing and variation testing is finally beginning to shine a few beams of light on the question that created this schism. In the end, it will not be surprising to find that both these antagonists were partly correct: some families may be transmitting blood pressure traits through single loci with large effects, while in the population at large blood pressure traits are arising through multiple loci with individual pedigrees transmitting effects from a limited number of such loci. In the end there may be satisfaction for both sides. But the important thing is to progress in this difficult challenge so that concrete and specific information about causes of hypertension is obtained that can reinvigorate and improve control of this highly prevalent and dangerous disease.

Conflicts of Interest

The author has no conflict of interest.

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