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COMMENTARY

Candidate genes revisited in the genetics of hypertension and blood pressure

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Despite considerable, concerted efforts over the past 20 years, it is only within the past 4 years that substantial progress has been made in identifying genetic variants robustly associated with blood pressure (BP) elevation or hypertension (Figure 1), largely due to technological advances.[1–3] We have witnessed great success in the identification of new susceptibility loci for BP and hypertension through genome-wide association (GWA) analysis. The number of robustly associated loci, that is, those that have attained a genome-wide significance level ($P<5\times10^{-8}$) and that have been repeatedly validated in independent samples, has increased to >40 today. Therefore, because GWA studies investigate a large number of single-nucleotide polymorphism (SNP) markers simultaneously, such a stringent statistical threshold should be set to avoid false positives from multiple testing.

Most loci associated with BP and/or hypertension map to regulatory or intergenic regions of the genome, and in many cases, the causal transcript remains undetermined. Surprisingly few of the GWA signals have mapped near strong biological candidates. Nevertheless, at certain loci, the causal transcript is inferred, based on a combination of supportive data, for example, coding variants (in particular, non-synonymous SNPs), nearby biological candidates and *cis* expression quantitative trait loci, which regulate mRNA expression levels. Among the loci harboring strong candidate genes, *ENPEP* (which encodes glutamyl aminopeptidase) and *NPR3* (which encodes natriuretic peptide receptor C/guanylate cyclase C) have been noted[4] because of their involvement in known BP regulatory pathways, that is, the renin–angiotensin–aldosterone system (RAAS) and natriuretic peptides. Despite the as-yet-undefined genetic architecture of complex traits, these findings suggest that molecular variants in some physiological candidate genes have a role in the etiology of BP elevation or hypertension. In this article, I will comment on the background and necessity of revisiting notable candidate genes in GWA analysis, as we have recently reported in this journal.[5]

In 2009, two consortia-based meta-analyses of GWA studies involving 30 000–40 000 samples in the discovery stage identified 13 independent loci that are significantly associated with BP in populations of European descent. Subsequently, meta-analyses of GWA studies in East Asians and in a larger consortium combining the Global BPgen and Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) consortia identified 21 additional loci. During these efforts, it has been widely recognized that for complex traits such as BP and hypertension, the majority of susceptibility loci may individually exert modest genetic effects, that is, a 5–20% increased risk of developing hypertension and an association between each copy of the risk allele and an average increase of approximately 1 mm Hg in the systolic BP or approximately 0.5 mm Hg in the diastolic BP.[2] Accordingly, these loci may not attain a genome-wide significance level unless a large number of individuals are analyzed in GWA scans.

Furthermore, it has been argued that the susceptibility loci identified to date through GWA studies explain only a small proportion of heritability (<3%) for BP. This discrepancy is termed 'missing heritability', as discussed elsewhere.[6] A question has been raised as to whether complex traits are affected by thousands of variants with small effects, but a recent analysis of GWA study data using a computational technique has suggested that many hundreds of common, weakly associated variants may be sufficient to account for the majority of heritability.[7] It is now assumed that although many true positive associations reside within GWA study data, most have not achieved a stringent threshold of statistical significance ($P<5\times10^{-8}$),[8] which is generally required for an association to be considered 'confirmed.' Accordingly, it is notable that the number of discovered variants (or loci) is strongly correlated with the experimental sample size in GWA studies of complex traits via incremental statistical power.[1] The power studies further suggest that larger GWA studies, with tens or hundreds of thousands of participants, will yield many more validated loci for complex traits, whereas these loci remain individually difficult to identify in current GWA studies with practically attainable sample sizes.[7] Besides statistical power, coverage of genetic markers in GWA scans may not always be sufficient to examine associations with particular functional variants (or mutations) in the target genes because during GWA studies, researchers normally assay only a subset of SNPs that can be chosen by considering the linkage disequilibrium structure in a given chromosomal region.[3]

Therefore, we conducted a large-scale association study of eight variants from seven candidate genes, namely, *ACE* (angiotensin-converting enzyme), *ADD1* (adducin 1), *ADRB2* (beta-2 adrenergic receptor), *AGT* (angiotensinogen), *CYP11B2* (aldosterone

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synthase), *GNB3* (G protein, beta-3 subunit) and *NOS3* (nitric oxide synthase 3), in the Japanese (in total, 21 851 samples from the Cardiometabolic Genome Epidemiology (CAGE) network study panels) and in a joint meta-analysis involving previously reported multi-study populations.[5] The individual contribution of each of these genes to BP variation and/or hypertension in the general population had not been thoroughly elucidated, although a number of studies had repeatedly investigated them as candidate genes. Our study demonstrated that BP-trait associations at two loci (*AGT* rs699 and *CYP11B2* rs1799998) were consistently replicated, and particularly, the hypertension association reached genome-wide significance ($P<5\times10^{-8}$) for these two variants.[5]

For the BP-trait-associated candidate gene variants, four features are noteworthy. First, the genetic impacts of individual candidate variants (or loci) are confirmed to be modest, in accordance with the findings from GWA studies; that is, the allelic odds ratios (ORs) for hypertension are mostly in the range between 1.10 and 1.24 for the variants presenting nominally ($P<0.05$) significant associations in the CAGE study panels.[5] This finding reflects the necessity of a large sample size to expose variants with smaller effects and partly explains why the strength of the BP-trait association in the previous reports has not reached a genome-wide significance level. Second, given the fluctuations in the strength of genetic associations observable among the relatively small studies, reproducibility should be assessed based not only on the data statistically summarized by meta-analysis but also on the data from multiple independent panels with a large sample size. In our study, although by current standards not sufficiently large in sample size, three independent panels ($n=1256$–$12\,569$) were used for the analysis, and the effect sizes have proven to be relatively consistent across the panels at the two associated loci; for example, OR = 1.24–2.04 for *AGT* rs699 and 1.22–1.59 for *CYP11B2* rs1799998 (risk allele homozygote *vs.* non-risk allele homozygote).[5] Third, the concordance of association between BP (that is, a quantitative trait) and hypertension (that is, a dichotomous trait) supports the claim of genetic effects on BP variation in the general population. In the 1990s, partly because of the feasibility of sample ascertainment, the candidate gene studies were designed and vigorously conducted as case–control comparisons, which are often liable to selection bias or population stratification. Again, for the two associated loci, namely, *AGT* rs699 and *CYP11B2* rs1799998, concordant associations with BP traits (systolic BP, diastolic BP and hypertension) have been found in the Japanese ($P=0.03$–1.5×10^{-5}) and in a joint meta-analysis ($P=0.01$–7.3×10^{-10}). Fourth, the strength of associations at these two candidate loci, estimated from the overall meta-analysis data, is almost comparable to that of a series of BP-trait loci identified via exploratory GWA scans.[5] However, it is probable that the associations were not followed up in the multistaged screening approach of the previous GWA studies principally because their statistical significance in the discovery stage did not attain a given threshold (for example, $P<1\times10^{-5}$), as above mentioned in the argument of heritability.

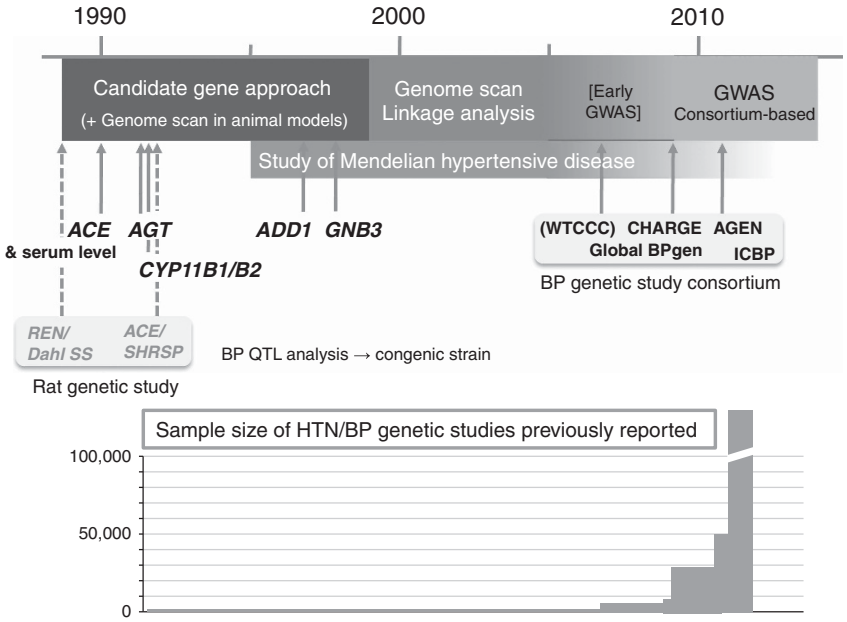

Figure 1 Progress in analytical approaches to the genetics of hypertension, with regard to the study sample size. In the 1990s, a number of candidate genes were repeatedly examined by case–control studies, but the sample size in the individual studies was mostly <1000. The sample size remained relatively small until the advent of GWA analysis (approximately 2007) and subsequently has increased rapidly over the past several years. A full color version of this figure is available at the *Hypertension Research* journal online.

I. Selection of target genes

Physiological candidate genes	Positional candidate genes
1) Genes in the known biological pathways, e.g., the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system (SNS)	1) Genes located in the linked region, which was detected by linkage analysis, e.g., affected sib-pair analysis
2) Novel genes identified in model organisms	2) Genes located in the associated region, which was detected by GWAS

II. Analytical approach

A selected set of variants	A comprehensive set of variants
Prioritize variants with some functional significance when testing genetic association at each target gene	Investigate a number of variants that can *tag* genetic polymorphism in the region harboring target gene(s)

Case-control study of hypertension	Quantitative (BP) association study
Diagnostic criteria should be clearly defined. The lack of population stratification should be validated.	BP values should be averaged for multiple measurements. For people under hypertension therapy, *imputed* BP values can be used.

Figure 2 Summary of the methodological considerations for conducting a candidate gene study in a comprehensive and systematic manner.

Several genetic variants tested in our study (at *ACE*, *AGT*, *CYP11B2*, *ADD1* and *GNB3*) had been repeatedly investigated as biologically plausible candidates (Figure 1) because of their physiological importance, that is, key molecules in the known BP regulatory pathways (for example, RAAS), causative genes in Mendelian hypertensive disease and novel findings supporting the possible contribution to BP regulation in model organisms. In addition, despite the lack of clear physiological evidence regarding BP elevation or hypertension, the GWA findings can further provide a list of positional candidate genes. These two categories of candidate genes—physiological and positional—must be examined in a more comprehensive and systemic manner as a target gene approach to complement the GWA scans (Figure 2). This analysis will help to elucidate the complex genetic architecture of hypertension.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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