Still Building on Candidate-Gene Strategy in Hypertension?
Frédérique Tesson, Frans H.H. Leenen

In the general hypertensive population, blood pressure (BP) is a multifactorial trait, and it appears that hypertension results from increments in BP from a number of contributing variants and their interactions with the environment. In spite of numerous linkage and association studies of candidate genes for BP per se, or BP response to salt, the genetic network responsible for BP variation remains elusive.

In recent years, several genome-wide scans have been performed in different populations around the world. The results have been rather variable, which can be attributed to different ethnic origins of the populations; often small sample sizes; lack of stratification by gender, age, and other determinants of BP; and limited assessment of the phenotype. However, 6 independent studies have reported evidence of linkage between systolic BP and chromosome 16p12 that includes SCNN1B and SCNN1G.1 In the present issue of Hypertension, Büsst et al1 focus attention on the influence of polymorphisms in SCNN1G, the gene encoding the γ-subunit of epithelial sodium channel (ENaC), on systolic BP. Three single nucleotide polymorphisms, considered individually or as a haplotype, appeared to be associated with systolic BP in logistic regression analyses adjusted for age, sex, and body mass index.1 The study was carried out in a white Australian population. This article is particularly attractive, because it confirms the involvement of “natural” susceptibility genes in BP levels. This is a good example of the importance of performing a comprehensive “gene-scan” analysis to determine whether a candidate gene is associated with the phenotype. Haplotype analysis refers to the simultaneous study of multiple alleles, as opposed to the study of single allele at a time. Although it is possible that the causal genetic variant is included in the set of variants/markers tested, the premise for this approach is that ≥1 of the variants tested will serve as a proxy for the causal variant. In this study, the number of polymorphisms analyzed per gene was sufficient to assume that the full extent of nucleotide sequence variation was captured.1

The BP response to salt is normally distributed with 2 extreme phenotypes, salt sensitive and salt resistant, and an intermediate phenotype. As many as 50% of patients with hypertension are salt sensitive in short-term studies compared with only ≈10% in the normotensive population. There are robust biologically relevant pathways involved in sodium (Na+) transport affecting BP response to salt. The majority of the genes from those pathways have been mapped to BP quantitative trait loci. Most of the regulation of Na+ transport occurs through ENaC and Na+/K+-ATPase. The pathogenic mechanisms of salt sensitivity are far from being completely understood; however, abnormal Na+ transport across epithelium of distal nephron and choroid plexus is likely to play a key role.

Direct evidence that ENaC dysfunction is involved in pathologic processes has come from the fact that all mutations identified in Mendelian forms of hypertension-related syndromes were found in genes involved in electrolyte transport functions, including SCNN1A, SCNN1B, and SCNN1G, encoding ENaC subunits.2-4 Rat models of salt-sensitive hypertension have pointed out the importance of the ENaC pathway in the molecular mechanisms mediating salt-induced hypertension not only in the kidney but also in the central nervous system.5 The Na+/K+-ATPase is involved in establishing and maintaining the electrochemical gradients of Na and K ions across the plasma membrane. The catalytic subunit of Na+/K+-ATPase is encoded by multiple genes. ATP1B1, which encodes a β subunit, is located in a BP quantitative trait locus for human, rat, and mouse, and variants in this gene are associated with differences in BP levels.6

Among the studied candidate genes, only the adducin 1 gene (ADD1) has been found to be significantly associated with salt-sensitive hypertension in several independent studies. Adducin, present in kidney and brain, is a heterodimeric cytoskeleton protein. Most recently, a new susceptibility gene for salt sensitivity, the chlorine channel CLC-Ka gene (CLCNKA), has been identified.7 Four CLCNKA single nucleotide polymorphisms were associated with the pressor response to an acute Na+ load.7 This study presents several design advantages compared with the present study. This is mainly attributable to the fact that, in the latter study, subjects were drawn from the Victorian Family Heart Study, established to address the cause of familial patterns in cardiovascular risk factors.1 Indeed, instead of assessing the influence of genotype in a never-treated essential hypertensive population, adjustment for treatment effects was used in the study published in this issue, because as much as 53% of the high systolic BP group was on antihypertensive medication.1 The comparison of this high systolic BP group with the low systolic BP group, in which the number of individuals under treatment was obviously much lower, may have introduced a statistical bias. Moreover, the BP response to any single drug is characterized by a large interpatient variation and, thus, is likely to be under genetic influence.

A major limitation for both genome-wide scans and association studies is the weak assessment of the phenotype. In
most studies, including the present one, BP is only measured by office measurements. These are notoriously variable or inaccurate and can readily misdiagnose, ie, overdiagnose “white-coat hypertension” or underdiagnose “masked hypertension,” leading to both false-positives and false-negatives. It is imperative that 24-hour ambulatory BP monitoring, the current “gold standard,” be included in the design of future protocols. At last, even in the absence of any known functional effect of the variants tested, a physiological link can be assessed for the association of the identified variants with intermediate phenotypes, such as electrolytes concentrations, plasma renin activity, aldosterone, or plasma norepinephrine.

Finally, there are several questions that remain to be addressed to understand the clinical significance and biological mechanisms by which these observations link the γ-subunit of ENaC and hypertension. To begin, the study needs to be replicated in larger independent populations. Association studies have become an increasingly popular approach to mapping variants that affect complex traits and common disease but are plagued by lack of reproducibility. The potential reasons for this lack of reproducibility essentially boil down to 3 causes: a false-positive association is correctly not replicated; a correct report of a true association fails to be replicated in an underpowered follow-up study (false-negative); a true association in one population is not true in a second population because of heterogeneity in genetic or environmental background. It should be noticed that the recent Wellcome Trust Case Control Consortium genome-wide association study identified several loci for 6 common diseases but failed to spot 1 for hypertension, perhaps because genuine common susceptibility variants of large effect size were poorly tagged by the set of single nucleotide polymorphisms genotyped. Another limitation of the study by Büsst et al is that it focused on only 1 gene while disregardng other genes within the same pathway and overlooking potential gene-gene interaction. If genes act together, the marginal effect of each gene might be small but might reflect much larger effects of collections of genes. Nevertheless, statistical evidence alone cannot distinguish between causal and nonfunctional variants in linkage disequilibrium with the causal polymorphism. Therefore, the evidence for causality must include compelling demonstration that this polymorphism can be translated into a phenotype of hypertension.

Should these observations be validated, the current findings have significant relevance to cardiovascular diagnostics and therapeutics. From an individual perspective, the impact of high salt intake on a person’s cardiovascular system can vary from minimal to substantial. The extent of this impact is clinically difficult to ascertain. Genetic diagnosis would be a method of choice for advising lifestyle interventions for a particular individual.

One of the main weaknesses of candidate-gene strategies is that they rely on a previous hypothesis, that the selection of candidate genes is limited to the best established biologically relevant pathways regulating BP levels, thus precluding the discovery of novel pathways. Concerns were raised previously whether the genome-wide association study design would be powerful enough to detect most genetic variants, with quite modest odds ratios (<1.25), involved in complex diseases. Because “proof of principle” that genome-wide association studies can be successful for common, complex phenotypes influenced by multiple genetic and environmental factors has now been demonstrated, such an approach is the only way to identify the multiple influencing genes, with each contributing a modest effect to the phenotype. Therefore, the next wave of interrogation of the genome for both single nucleotide polymorphisms and copy number variations may be an effective way to elucidate the causes of BP dysregulation and allow us to ultimately translate genetic variants into personalized genetic medicine.

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### References
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