Investigations of the genetic underpinnings of hypertension have had a long history that includes twin studies, adoption studies, and family studies. Subsequent large pedigree studies showed substantial heritability for many traits related to hypertension. Guided by these high heritabilities, segregation analysis provided evidence that common mendelian genes had moderate to large effects on multiple intermediate phenotypes for blood pressure and for blood pressure itself. Subsequent linkage analyses suggested that these large common gene effects were not as strong as originally thought because few linkage signals reached the strength seen for known monogenic traits. The excitement surrounding the potential of whole-genome linkage scans seemed to diminish because few scans agreed on the same chromosome regions. However, as more scans have been published and more meta-analyses performed, increasing consensus exists for particular chromosomal regions containing genes contributing to blood pressure control (see the National Heart, Lung, and Blood Institute GENELINK meta-analysis Web site, https://genelink.nhlbi.nih.gov/index.jsp). A number of genes have now been discovered by fine mapping regions under linkage peaks (SLC4A5, ATP1B1, SELE, RGS5), suggesting that despite the lack of universal agreement of multiple scans on linked regions and only moderately sized linkage statistics, it is still possible to find disease genes under linkage signals. In addition to the recent gene-finding successes of family studies, whole-genome association studies have become the new hope to discover additional genes with small but important effects on blood pressure. Genome-wide association studies have now identified genes or specific chromosomal locations influencing many diseases such as diabetes mellitus, obesity, and coronary heart disease. Additional association scans now in progress will surely identify other disease genes, including new blood pressure genes. A genome-wide association study consisting of 2000 subjects for each of 7 diseases and 3000 control subjects suggested 6 single-nucleotide polymorphisms (SNPs) associated with hypertension with probability values ranging from $10^{-5}$ to $10^{-7}$ before adjustment for the number of statistical tests. These 6 SNPs should be followed up in other samples so that the severe penalty of correcting the probability values for a large number of correlated statistical tests can be removed. Even if 1 of these 6 SNPs or other SNPs from other genome scans are confirmed, multiple association studies will probably show inconsistencies of positive findings as did the candidate gene and linkage studies. Any 1 study will not detect all hypertension genes because detection of a gene association in a particular sample depends on subject ascertainment and ancestry, density and selection of markers, phenotypes and methods of measurement, subtle differences in methods of analysis, size of the effect, and random error. Even though the FTO gene was associated with obesity with a significance of $10^{-35}$, it explained only $\approx 1\%$ of the population variance in body mass index and could easily be missed in other samples. None of the 6 association regions for hypertension in the above study overlap with candidate genes previously suggested to be associated with hypertension through a candidate gene or positional candidate gene approach after linkage. In particular, candidate genes already with substantial replication and physiological understanding of the genetic defect such as angiotensinogen (AGT), $\alpha$-adducin (ADD1), $\beta$-adrenergic receptor (ADRB2), G protein $\beta$ subunit polymorphism (GNB3), aldosterone synthase (CYP11B2), or dopamine-related receptor (GRK4) were not in any of the regions detected in the hypertension scan. Lest one become too enamored of a particular method of discovering hypertension genes, the above examples clearly show that multiple approaches are needed. Continued follow-up of linkage peaks, whole-genome association studies, and candidate gene investigations should all continue. Each has its own strengths and weaknesses. The gene expression and proteomic approaches will be useful additions to the genetic studies. Multiple approaches should help us discover what factors prevent detection of association in some populations, giving hints to gene–environment and gene–gene interaction. Interactions will play a pivotal role in our understanding of the pathophysiology of the complicated blood pressure control systems involved with the development of hypertension.

In this issue of Circulation, Rao et al have taken a standard candidate gene approach to detect genes for intermediate phenotypes related to blood pressure. The tyrosine hydroxylase gene (TH) is an obvious candidate for catecholamine control of blood pressure, which, compared with genetic studies on the renin-angiotensin-aldosterone system, has been understudied. The authors have taken a comprehensive approach to investigate these phenotypes by first rese-
quencing a prime candidate gene, \( TH \), which is the rate-limiting step in catecholamine synthesis. A tetranucleotide repeat had already been identified in intron 1 of the \( TH \) gene\(^{16} \) and had been shown to have some transcriptional regulation functions.\(^{17} \) This repeat was associated with norepinephrine levels and hypertension,\(^{18} \) and the association was later confirmed.\(^{19} \) However, the present study represents the first attempt to completely characterize \( TH \) gene variability to detect functionally important polymorphisms.

After resequencing, the authors studied the relationship of the identified SNPs with urine and plasma catecholamine levels and baroreceptor function, which have been suggested as important intermediate phenotypes for blood pressure control. Single SNP analysis was followed by haplotype analysis to improve the genetic information on each subject for the \( TH \) gene. Despite 4 SNPs belonging to a haplotype block in the promoter region of the gene, only 1 SNP was strongly associated with the phenotypes, with marginal associations of 2 other SNPs with \( \pm 1 \) phenotypes. Haplotype analysis did not particularly increase power over use of the C-824T SNP alone to help define a smaller subgroup at risk for hypertension. The associations found for the \( TH \) gene were with SNPs in the promoter region, not in a coding region. This seems to be a frequent feature of many of the common genes proposed to contribute to hypertension. No evidence existed for the 2 common or any of the rare coding SNPs being associated with any of the phenotypes.

Once the associations of the intermediate phenotypes were established, resting blood pressure and stressed blood pressure induced by a cold pressor test were studied to help verify that the significant effect of the \( TH \) gene on the intermediate phenotypes was detectable on the end points of interest, a test of pleiotropy. Stressing a system with defective control often will exaggerate the underlying abnormalities, allowing easier identification. The evidence presented suggests that catecholamines and blood pressure both have some genetic control by the \( TH \) gene. The percent of variance explained by \( TH \) for catecholamines and blood pressure ranged from 1% to 9%, which is on the order of the percent of variance explained to date by other hypertension genes with common alleles.

The statistical studies were followed by functional assays to determine whether specific SNPs or haplotypes showed altered transcription rates or resulted in increased levels of catecholamine production. These studies showed that specific haplotypes did have transcription effects on \( TH \) and on increased catecholamine levels. Still to be elucidated is whether the suggested transcriptional effects of the microsatellite in intron 1 are independent of the promoter haplotype effects.

Because multiple initial reports of disease-related genes have not subsequently been replicated or were replicated only in some studies, it is important that replication accompany initial reports of association. The authors included a replication sample in their study and confirmed their main association findings, providing strong motivation for continued study of these phenotypes, which are part of an important blood pressure pathway.

This study illustrates the difficulties of investigating interactions in that the interactions found were mostly barely significant and the interaction of sex and genotype on blood pressure was not replicated in the second sample of subjects. Nonreplication may have occurred because the ascertainment criteria were different, so it was not a true replication sample. Another difficulty was that even though baroreceptor function is part of the pathway involving catecholamines and blood pressure, the results were not convincing that baroreflex activity was related to \( TH \). Although some evidence existed for interaction of baroreceptor function with \( TH \) haplotypes on urine norepinephrine, the sample size of 32 was quite small for the 1 haplotype group that seemed to deviate from the other 2 haplotype groups. Interaction studies are always difficult because of the requirements of very large sample sizes needed to get sufficient numbers in the minor allele genotype or less common haplotype groups for adequate testing. These secondary findings of this study need to be investigated further now that evidence for consistent relationships between \( TH \) and catecholamines has been found.

The variance of intermediate phenotypes explained by a gene variant is likely to be larger than published estimates. If one could study the effects of the \( TH \) locus in the absence of the normal counterregulation that exists when 1 physiological component changes, the effects may be much larger. The redundant counterregulatory mechanisms are powerful, although they appear to lose effectiveness with age. These mechanisms may fail to fully counterregulate the genes involved in blood pressure control, and the apparent genetic effects of any 1 gene may appear or become stronger with aging. Of course, obesity, lack of exercise, and a poor diet may have strong regulatory effects on biochemical/hormonal phenotypes and blood pressure and may overwhelm the small to moderate genetic effects of any 1 gene. As a side note, the complicated gene–environment interactions on blood pressure that are so difficult to define at present may be best represented by simply calculating a person’s family history of hypertension. Family history models the incidence/prevalence of hypertension in a family rather than individual risk factors and is currently a strong, independent determinant of hypertension incidence in unaffected family members.\(^{20} \) It is an independent risk factor because it captures the results of the risk factors and their interactions without a need to model those complicated and unknown interactions.

Although further replication studies of the \( TH \) locus and catecholamine pathophysiology are needed, this study provides another possible gene that can be added to the collection of genes that increase the risk of hypertension. All hypertensive subjects will not have the same set of interacting genes contributing to their hypertension. The more hypertension genes that can be identified, the more the various combinations of genes can be identified in particular subsets of subjects. However, the more pieces of the hypertension puzzle that we discover, the larger the study sample sizes need to be to figure out how to put the puzzle together. As we get closer to reaching that goal, improved targeted therapies developed from specific puzzle pieces will likely become available to improve control of high blood pressure and possibly to prevent hypertension and resultant cardiovascular disease.

**Disclosures**

None.
References


Tyrosine Hydroxylase: Another Piece of the Genetics of Hypertension Puzzle
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