Genetic Variants of WNK4 in Whites and African Americans With Hypertension

Porat M. Erlich, Jing Cui, Irmarie Chazaro, Lindsay A. Farrer, Clinton T. Baldwin, Haralambos Gavras, Anita L. DeStefano

Abstract—Human chromosome 17q has been implicated to contain a gene that influences hypertension susceptibility. This region contains the WNK4 gene that causes the mendelian disorder pseudohypoaldosteronism type II, characterized by high potassium levels and hypertension. The goal of this study was to identify genetic variants in all exons of WNK4 in hypertensive individuals and to examine the association of these variants with essential hypertension. Single-nucleotide polymorphisms (SNPs) were identified by sequencing the entire coding region in 32 whites and 32 African Americans with hypertension. A single SNP in whites and 8 SNPs in African Americans were genotyped in a larger cohort of whites (165 hypertensives; 91 normotensives) and African Americans (120 hypertensives; 98 normotensives). The frequency of the rare allele differed significantly between hypertensive whites (13.0%) and normotensive whites (7.1%, \(P=0.040\)) for the single intronic SNP (bp 1 156 666). This difference remained significant after adjusting for body mass index and sex (\(P=0.035\)). Genotypic frequencies differed significantly between hypertensive and normotensive individuals when a dominant model either with (\(P=0.027\)) or without (\(P=0.028\)) covariate adjustment was assumed. The odds ratio for hypertension was 2.28 for AA or AG individuals vs those with the GG genotype (95% confidence interval, 1.09 to 4.75). No significant differences in allelic or genotypic frequencies were observed in African Americans for any SNPs. The finding in whites is consistent with the hypothesis that polymorphisms in WNK4 influence the risk of hypertension. However, because the associated SNP does not appear to be a functional variant and the limitations of case/control association studies, confirmation of these results in additional cohorts is warranted. (Hypertension. 2003;41:1191-1195.)

Key Words: gene expression ■ hypertension, essential ■ genetics ■ polymorphism

The prevalence of hypertension is \(\approx35\%\) in adult African Americans and \(\approx20\%\) in white Americans, and because of the associated morbidity and mortality, hypertension presents a major public health problem. Quantitative measures of blood pressure and clinical phenotypes, such as hypertension, demonstrate a genetic component. Searches for genetic factors that influence blood pressure or susceptibility to hypertension have often led to conflicting results (eg, association studies of the angiotensin-converting enzyme variants) or irreproducible results (eg, evidence of linkage to a specific chromosomal region).

Several lines of evidence implicate a region on human chromosome 17q as harboring a gene that influences blood pressure. This region was first identified in studies of the spontaneously hypertensive rat, in which a quantitative trait locus (QTL) on rat chromosome 10, syntenic to human chromosome 17, was localized.\(^7\) Subsequently, evidence of linkage with hypertension in this region was demonstrated in hypertensive sib pairs from the United Kingdom and France\(^8\) and was confirmed in a study of white American hypertensive sib pairs.\(^4\) However, linkage was not observed in African American hypertensive siblings.\(^4\) Significant evidence of linkage was also observed in a study of blood pressure in the Framingham Heart Study, a population-based sample. A logarithm of odds (LOD) score of 4.7 at 67 cM on chromosome 17 was observed for systolic blood pressure (SBP).\(^5\) In contrast, other genome-wide scans have not demonstrated linkage to this blood pressure QTL.\(^6,7\)

This region on human chromosome 17q21.2 contains the gene for WNK4, a serine-threonine kinase that comprises 19 exons contained within a 16-kb length of genomic DNA. Mutations in WNK4 have been shown to cause the rare autosomal dominant disorder pseudohypoaldosteronism type II (PHA2),\(^6\) which is characterized by high potassium levels (hyperkalemia) and hypertension (OMIM 145260). WNK4 expression localizes to the distal convoluted tubules of the kidney and specifically to the intercellular junctions. This expression pattern, along with the clinical features of PHA2,
suggests that WNK4 plays a role in regulating the balance between Cl⁻ reabsorption and K⁺ and H⁺ secretion. This hypothesis is supported by evidence that WNK4 inhibits the function of the Na-CI cotransporter (NCCT) by negative regulation of NCCT surface expression. NCCT regulates the reabsorption of Na⁺ with Cl⁻, and the inhibitory function of WNK4 is lost in variants containing mutations observed in PHA2. Although there might be additional targets of WNK4, the inhibition of NCCT might explain the molecular pathogenesis of PHA2 and suggest a mechanism by which other variants in WNK4 might influence susceptibility to essential hypertension. Given that polymorphisms in WNK4 are causative mutations in a genetic disorder characterized by hypertension, this gene is an obvious leading candidate for influencing blood pressure and increasing susceptibility to hypertension, this gene is an obvious leading candidate for influencing blood pressure and increasing susceptibility to essential hypertension among the large number of genes (at least 140) located in this region of chromosome 17.

The goal of this study was to identify genetic variants in all exons of WNK4 in a group of hypertensive whites and African Americans and to examine the association of these variants with essential hypertension. In contrast to other association studies, which often focus on a limited number of polymorphisms in a gene, our study evaluated the full array of coding-sequence polymorphisms in WNK4.

### Methods

#### Subjects

Unrelated patients with essential hypertension were identified from the hypertension clinics at the Boston Medical Center, Boston Mass. All subjects provided informed consent, and the study was approved by the human subject review board at Boston University School of Medicine. Individuals were classified as hypertensive if pretreatment SBP was ≥140 mm Hg, pretreatment diastolic blood pressure (DBP) was ≥90, or the individual was being treated for hypertension. A routine clinical evaluation was carried out and, whenever clinically appropriate, additional standard diagnostic procedures were conducted to exclude organic causes of secondary hypertension (eg, renovascular lesions, adrenal tumors). Ethnically matched, normotensive subjects (SBP<130 mm Hg, DBP<80 mm Hg, and not being treated with antihypertensive medications) >55 years old were recruited from the same populations from which the hypertensive individuals had been ascertained. There were a total of 285 hypertensives (165 whites, 120 African Americans) and 189 normotensive controls (91 whites, 98 African Americans).

#### Sequencing

Initial SNP identification was performed by sequencing the entire coding region of 32 hypertensive African Americans and 32 whites. Primers flanking the coding region were used in a polymerase chain reaction (PCR) to generate a sequencing template (Table 1). After cleaning the product (QiAquick 96 PCR purification kit; Qiagen), a sequencing reaction (BigDye terminator V. 3.0 cycle sequencing kit, ABI) was performed with nested primers. The reaction was cleaned (DyeEx 96 spin kit; Qiagen) and run on a DNA analyzer (Applied Biosystems 3700). Although the main target of sequencing was the coding region, the primers used also covered some intronic sequence. Hence, some intronic SNPs are reported in addition to coding-region SNPs.

Polymorphisms were further investigated when variant alleles were observed in at least 2 individuals or were present in a public database. Of the SNPs detected by sequencing, only 1 (P896P) was listed in http://www.ncbi.nlm.nih.gov/SNP/ and is identified as SNP rs2290042. Given our sample size of 32 individuals of a specific ethnic group for sequencing, this strategy yielded a 99% probability of detecting a SNP with a frequency of 0.10 and a 84% probability of detecting a SNP with a frequency of 0.05. Although the probability of detecting very rare alleles (frequency <0.04) was ~50%, such alleles are not likely to be major susceptibility alleles for a common disorder. For polymorphism detection in the larger sample of hypertensives and controls, either DNA sequencing (see previous paragraph) or homogeneous MassEXTEND (Sequenom, Inc) was used. Homogeneous MassEXTEND is based on mass
spectrometry detection of differences in primer extension products due to the presence or absence of a polymorphism in the template DNA.

Statistical Analysis
Allelic and genotypic frequencies were compared between cases and controls by using a χ² test or the Fisher exact test when appropriate. These tests do not account for covariates such as sex and body mass index (BMI), which might influence the risk of hypertension. Logistic regression analysis was used to compute the odds ratio (OR) for hypertension based on genotype after accounting for the covariates sex and BMI. Although the risk for hypertension is also age dependent, age was not included as a covariate, because the normotensive individuals were selected by an age criterion to account for the age-dependent penetrance of hypertension. For all genotypic analyses, different genetic models were specified, including a general model (each genotypic effect is estimated independently), an additive model (assumes that the heterozygote effect is the average of the 2 homozygotes), and a dominant model (assumes that possession of 1 rare allele has the same effect as being homozygous for that allele).

To assess the OR for a specific allele, each individual can contribute 2 observations to the analysis because each person possesses 2 alleles. A generalized estimating equation was implemented to account for nonindependent observations in the allelic model when adjusting for the covariates sex and BMI. Genotypic and allelic analyses were conducted with SAS, version 8.2.

The program Arlequin was used to estimate and compare haplotype frequencies between hypertension and normotensive groups. Haplotypes cannot be unambiguously determined for completely homozygous individuals or for individuals heterozygous at 1 polymorphism and homozygous at the remaining polymorphisms. The expectation-maximization (E-M) algorithm is implemented in Arlequin to estimate the haplotype frequencies by using the genotype information from all individuals, even when their haplotypes cannot be unambiguously determined. The E-M algorithm is an iterative procedure in which the 2 steps, the E step and the M step, are alternated until the change in estimated haplotype frequencies is so small from the prior iteration of E and M steps that the algorithm is said to converge. In this specific implementation, initial estimates of haplotype frequency are randomly chosen, and the E step consists of computing the expected genotype frequencies based on that haplotype distribution and with the assumption of Hardy-Weinberg equilibrium. In the M step, the new genotype frequencies are used as weights in a gene-counting procedure to obtain new estimates of haplotype frequencies.

To test for differences between the haplotype distribution in normotensive individuals and hypertensive individuals, we computed the exact test of population differentiation as implemented in Arlequin. This test is similar to Fisher’s exact test for a 2×2 contingency table extended to a k×k contingency table, where k is the number of distinct haplotypes observed in the 2 populations. Statistical significance is assessed by using a Markov chain to evaluate all possible states of the contingency table and estimating the probability of observing a table less or equally likely than the observed haplotype frequencies under the null hypothesis that the 2 samples (hypertensive and normotensive individuals, in this case) are from the same population.

Results
A striking difference in the degree of variability of WNK4 was observed between African Americans and whites. After sequencing a panel of 64 hypertensive individuals (32 African Americans and 32 white Americans), only 1 polymorphism (intron 10 SNP) was identified in the whites, whereas 8 variants were observed in the African Americans. Of these polymorphisms, 6 resulted in codon changes, including 4 predicted amino acid changes: A589S results in substitution of a nonpolar by a polar residue; R665W results in substitution of a positively charged residue by a bulky aromatic residue; and P813L and T879M are conservative with respect to major residue characteristics (Table 2). We did not detect any mutation that has previously been shown to cause PHA2.

We next typed each of the 8 polymorphisms discovered from sequencing 64 individuals in a larger cohort of African American and white cases and controls (474 individuals total) and evaluated their association with hypertension. For the single polymorphism present in whites, we observed a significant difference in allelic frequencies (P=0.04) between the patients with hypertension and normotensive controls (Table 3). The rare allele (A) had an observed frequency of 13.0% in hypertensive individuals and 7.1% in normotensive individuals. Genotypic differences between the 2 groups were not significant when all 3 genotypic classes were considered (P=0.056). However, if one assumes a dominant model in which individuals with at least 1 A allele (AA and AG individuals) are combined, we observed a significant difference between normotensive and hypertensive individuals (P=0.03), suggesting that the GG genotype might be protective against hypertension. However, no association was observed for this polymorphism in African Americans (Table 4).

The association between hypertension and the SNP observed in whites was further explored by adjusting for the covariates sex and BMI by use of a logistic regression model.
A significant association between the SNP genotypes and hypertension was observed after accounting for sex and BMI and assuming either a dominant model ( \( P = 0.028 \)) or an additive model ( \( P = 0.037 \)). The genotypic effects were not significant in the general model ( \( P = 0.09 \)) when adjusting for covariates. By implementing the dominant model, the OR for hypertension was 2.28 (95% confidence interval [CI], 1.09 to 4.75) for individuals with at least 1 A allele compared with individuals with 2 G alleles. Allelic association also remained significant when adjusting for covariates ( \( P = 0.035 \)) and yielded an OR for hypertension of 2.07 (95% CI, 1.05 to 4.07) for the A allele compared with the G allele.

For the remaining variants discovered by sequencing in African Americans, there were no differences in allelic or genotypic frequencies between hypertensive individuals and controls (Table 4). No association was detected for any genetic model or after adjustment for covariates (results not shown). The allelic frequency of the rare allele ranged from 2% to 24% for the various SNPs.

Not surprisingly, there was a strong linkage disequilibrium between the multiple SNPs within WNK4 observed in African Americans. Of the 256 possible haplotypes based on 8 SNPs, only 10 were observed in the total sample. The estimated frequency of the haplotype consisting of the most common allele from each SNP was 0.68 in the combined sample of normotensive and hypertensive African Americans, which is higher than the 0.38 expected on the basis of allele frequency if one assumes that all SNPs were segregating independently. Haplotype analyses with all markers simultaneously (Table 5) revealed no significant difference between hypertensives and normotensives for African Americans ( \( P = 0.30 \)).

**Discussion**

Given the evidence of linkage of the chromosomal region containing WNK4 to essential hypertension and the observation that WNK4, when defective, causes PHA2, this gene is a strong candidate for hypertension susceptibility. To test this hypothesis, we sequenced the entire coding region of the gene.
TABLE 5. Estimated Haplotype Frequencies Based on All 8 SNPs in African Americans*

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Hypertensives</th>
<th>Normotensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCGGCGCGCG</td>
<td>0.068</td>
<td>0.067</td>
</tr>
<tr>
<td>CTCGCCCGCC</td>
<td>0.016</td>
<td>0.020</td>
</tr>
<tr>
<td>CTCGCCGC</td>
<td>0.649</td>
<td>0.713</td>
</tr>
<tr>
<td>TCTGGTCA</td>
<td>0.003</td>
<td>0.004</td>
</tr>
<tr>
<td>TCTTGGCAA</td>
<td>0.004</td>
<td>0.003</td>
</tr>
<tr>
<td>TTTCATCA</td>
<td>0.004</td>
<td>Not observed</td>
</tr>
<tr>
<td>TTTGCTAA</td>
<td>0.096</td>
<td>0.027</td>
</tr>
<tr>
<td>TTTGCCTA</td>
<td>0.034</td>
<td>0.029</td>
</tr>
<tr>
<td>TTTGCTCA</td>
<td>0.102</td>
<td>0.093</td>
</tr>
<tr>
<td>TTTTGGCAA</td>
<td>0.023</td>
<td>0.044</td>
</tr>
</tbody>
</table>

*There is no significant difference in the haplotype distribution between the hypertensive and normotensive individuals based on the exact test of population differentiation (P=0.30).

in a series of 32 hypertensive whites and 32 hypertensive African Americans and evaluated the association in a larger cohort of cases and controls. Eight polymorphisms in African Americans and 1 in whites were identified. This difference in genetic variability is consistent with genetic diversity in people of African origin.

For the single SNP observed in whites, the rare allele had an increased frequency in hypertensives compared with normotensives, suggesting an association with hypertension status. Although this SNP was observed in African Americans, the mutated allele was rare (4%) and did not differ by hypertensive status. These findings are consistent with a previous study in these same populations, which found linkage to the WNK4 region of chromosome 17 in white hypertensive sib pairs but not in African Americans. The unrelated cases in this study included 1 member of each affected sibship previously used in linkage analyses, along with additional probands.

The SNPs found to be associated in the white population is intrinsic (base 1156666 on human chromosome 17) and has no known or predicted functional effect on the WNK4 protein. Hence, it is unlikely that this SNP directly confers susceptibility to hypertension. Rather, the SNP may be in linkage disequilibrium with a functional polymorphism in a portion of the gene not examined by sequencing. We did not detect any coding region changes, which suggests that a functional polymorphism in WNK4 must be either in the 5' or 3' untranslated regions (eg, a promoter polymorphism affecting transcription) or in an intron (eg, abolishing or creating a new splice site). Alternatively, this SNP might be in linkage disequilibrium with a functional polymorphism in a neighboring gene.

The data presented here support the hypothesis that mutations in WNK4 confer susceptibility for essential hypertension in whites. However, we consider this finding to be preliminary, because it is based on a single intrinsic SNP and because of the limitations of association studies in unrelated individuals. Although the normotensive subjects were selected from the same geographic and ethnic group as the hypertensive individuals, we cannot exclude the possibility that population admixture has resulted in a false-positive association between WNK4 and hypertension. Additional studies that include additional sequencing of the WNK4 gene, association studies in other white populations, and family-based association tests are required to confirm the role of WNK4 in hypertension.

Acknowledgments

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References

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