Association of WNK1 Gene Polymorphisms and Haplotypes With Ambulatory Blood Pressure in the General Population

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Background—Blood pressure (BP) is a heritable trait of major public health concern. The WNK1 and WNK4 genes, which encode proteins in the WNK family of serine-threonine kinases, are involved in renal electrolyte homeostasis. Mutations in the WNK1 and WNK4 genes cause a rare monogenic hypertensive syndrome, pseudohypoaldosteronism type II. We investigated whether polymorphisms in these WNK genes influence BP in the general population.

Methods and Results—Associations between 9 single-nucleotide polymorphisms (SNPs) in WNK1 and 1 in WNK4 with ambulatory BP were studied in a population-based sample of 996 subjects from 250 white European families. The heritability estimates of mean 24-hour systolic BP (SBP) and diastolic BP (DBP) were 63.4% and 67.9%, respectively. We found statistically significant (P<0.05) associations of several common SNPs and haplotypes in WNK1 with mean 24-hour SBP and/or DBP. The minor allele (C) of rs880054, with a frequency of 44%, reduced mean 24-hour SBP and DBP by 1.37 (95% confidence interval, −2.45 to −0.23) and 1.14 (95% confidence interval, −1.93 to −0.38) mm Hg, respectively, per copy of the allele.

Conclusions—Common variants in WNK1 contribute to BP variation in the general population. This study shows that a gene causing a rare monogenic form of hypertension also plays a significant role in BP regulation in the general population. The findings provide a basis to identify functional variants of WNK1, elucidate any interactions of these variants with dietary intake or with response to antihypertensive drugs, and determine their impact on cardiovascular morbidity and mortality. *(Circulation. 2005;112:3423-3429.)*

Key Words: blood pressure ■ genetics ■ hypertension ■ kidney ■ risk factors

Blood pressure (BP) is a key determinant of cardiovascular health.1,2 Familial aggregation of BP has long been recognized, and estimates of the heritability of systolic (SBP) and diastolic (DBP) BP have exceeded 50%.3,4 The identification of genes involved in BP regulation, by improving knowledge of the relevant biology, should facilitate advances in treatment and control of BP. However, BP is a complex trait, and genetic studies into its etiology are constrained by the small effect sizes of the individual genetic variants, imprecise measures of the phenotype, and low-power approaches to study design and analysis.

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Studies of monogenic syndromes have provided important insights into a number of mechanisms underlying BP regulation.5 Although these provide evidence for a causal relation between gene and disease, none of these genes have yet been shown to directly affect BP in the general population.5 Recently, mutations in the WNK1 and WNK4 genes, which encode proteins in the WNK (“with no lysine” [K]) family of serine-threonine kinases, have been shown to cause pseudohypoaldosteronism type II (PHAII, or Gordon’s syndrome), an autosomal-dominant condition characterized by hypertension and hyperkalaemia.6 The WNK1 and WNK4 proteins localize to distal nephrons, WNK1 normally inhibiting the Na-Cl cotransporter in the apical membrane of epithelial cells lining the distal convoluted tubule.7,8 Thus, “gain-in-function” mutations in WNK1 or “loss-of-function” mutations in WNK4 result in PHAII that involves Na-CI cotransporter overactivity.7,8 The Na-CI cotransporter is sensitive to thiazide diuretics, and patients with PHAII exhibit an unusually large BP fall in response to thiazides.9

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WNK1 spans 156 kb of genomic DNA, with at least 28 exons producing multiple transcripts owing to alternative splicing, and is highly polymorphic, with >100 validated single-nucleotide polymorphisms (SNPs) (dbSNP, build 124; available at http://www.ncbi.nlm.nih.gov/projects/SNP/; last accessed February 11, 2005). WNK4 spans 16 kb of genomic DNA and 19 exons and, in white European subjects, is much less polymorphic, with only 1 common SNP reported (11566666G→A).10

A few studies have examined the association of polymorphisms in WNK1 and/or WNK4 with the risk of hypertension.10–13 A study in Japanese subjects found a nominal association with hypertension and 1 WNK4 SNP (C14717T).11 Although an association between another WNK4 SNP (11566666G→A) and hypertension has been reported in white American subjects,10 this finding was not replicated in a study of white Australian subjects,12 but both studies were small. Recently, the BRIGHT study provided evidence supporting a possible association between a WNK1 SNP (rs1468326) and the severity of hypertension in a family study of extremely hypertensive white European subjects.13 However, no study has yet examined whether common variants in WNK1 or WNK4 are associated with BP regulation in a population-based sample that has not been selected for the presence or absence of hypertension.

The main objective of our study was to investigate the association between common SNPs in WNK1 with BP in a population-based sample. Because BP measured at a single time is subject to transient variation and because BP exhibits circadian variations, we measured ambulatory BP for 24 hours to characterize it more precisely and to maximize the power to detect genetic determinants with a modest effect on BP. For completeness, we also included in our investigation the single WNK4 SNP observed in white European populations.10

Methods

Subjects and Phenotyping

We studied 1005 white European subjects from 252 nuclear families recruited from the general population in the ongoing GRAPHIC (Genetic Regulation of Arterial Pressure of Humans in the Community) study.

Families were included if both parents aged 40 to 60 years and 2 offspring ≥18 years wished to participate. Families were recruited by writing to women aged 40 to 59 years who had registered with their family practitioners in Leicestershire, England, inviting them and their families to take part. Subjects were excluded if they had renal disease or a comorbidity that affected accurate BP measurement. There was no preferential selection based on history of hypertension. Interviews by research nurses consisted of a detailed history and examination, including clinic BP and collection of blood samples. The Leicestershire Research Ethics Committee approved the study, and all subjects provided written, informed consent.

Ambulatory BP was measured with a Spacelabs 90207 monitor (Spacelabs) for 26 hours. The first 2 hours of each record were discarded to avoid any alerting response. The ambulatory monitor recorded BP at 30-minute intervals between 8 AM and 9:59 PM (“daytime”) and at 1-hour intervals between 10 PM and 7:59 AM (“nighttime”). If ambulatory BP profiles were <80% complete, they were repeated. We summarized the ambulatory BP data, weighting each time period proportional to its length. The 6 phenotypic outcomes on which our analyses focused were the time-weighted-

Structure of the human WNK1 gene and location of the SNPs analyzed. Exons are shown as vertical lines. Alternatively spliced exons are unfilled. The locations of the SNPs typed are indicated by arrows and are as follows: (1) rs1468326, (2) rs2368402, (3) rs765250, (4) rs2286007, (5) rs880054, (6) rs956868, (7) rs953361, (8) rs2301880, and (9) rs2260208.

means of SBP and DBP for 24 hours, during the daytime, and during the nighttime. Further details of recruitment and phenotyping, including response rates and measurement of clinic BP, are included in the online-only Data Supplement.

Genotyping and Quality Assurance

We genotyped 9 WNK1 SNPs (the Figure). The choice of SNP was informed by the results of a prior analysis of WNK1 described elsewhere.13 In brief, 19 SNPs spanning WNK1 at ~10 kb and including all nonsynonymous coding SNPs and the SNPs immediately 3' and 3' of the gene were typed in a separate population of 100 families.11 The 9 SNPs were chosen in an initial tag SNP (tSNP) selection by eye. In a more detailed analysis with established criteria,14 8 of these SNPs (excluding rs2301880) were found to be sufficient to predict common WNK1 haplotypes (minimum R2 = 100%, R2 = 94%) with >90% power.13 However, we present data on all typed SNPs, including rs2301880. The WNK4 (11566666G→A) SNP was also typed. Genotyping for the SNPs was done by fluorescent allelic discrimination with the ABI Prism 7900HT sequence detector system (Applied Biosystems). Details are given in the Data Supplement.

To assist in the identification of misclassified family relationships, we also genotyped 3 highly polymorphic microsatellite markers, d19s220, d3s1267, and d4s412, from the ABI Prism linkage mapping set v2.5-MD10 (PE, Applied Biosystems). Details are given in the Data Supplement. We checked for misspecified family relationships with the PedCheck program.15 Simulation analysis in 1000 families showed that the 3 microsatellites provided >99.3% power to detect any mendelian inconsistency. Two complete families and 1 individual (total of 9 subjects) were excluded after showing mendelian inconsistencies. In the remaining 996 subjects from 250 families, using PedCheck we then searched for mendelian inconsistencies in the SNP data attributable to genotyping error. The SNP data that were inconsistent with mendelian inheritance (13 observations) were coded as missing.

Statistical Analysis

Departure from Hardy-Weinberg equilibrium was tested with a χ² test on parental SNP data. We estimated ID1 and R² measures of linkage disequilibrium between pairs of SNPs with the JLIN program.16 Estimates of variance components, heritability, and the effects of individual SNPs were obtained by fitting generalized linear mixed models (GLMMs) using Gibbs sampling in WinBUGS.17,18 These models deal appropriately with the correlation of traits, genotypes, and environmental exposures within families.17,18 A censored normal approach19 was used to adjust for the effect of antihypertensive therapy. A GLMM was fitted to estimate narrow-sense heritability for each ambulatory BP phenotype, including age and sex (but no genes) as covariates. The SNP covariates were then included in the model, 1 at a time, and the effect of each SNP was estimated under an additive genetic model. Although the GLMMs were fitted with a Bayesian approach, flat prior distributions were used throughout, and inferences are reported as probability values and 95% confidence intervals (CIs).20 Using the 8 tSNPs, we also undertook a test of association of WNK1 haplotypes with BP phenotypes in the presence of linkage by
using HBAT. The input values for HBAT were the residuals from a normal linear regression correcting for age and sex as covariates and adjusting for treatment effects using a nonparametric algorithm.4 Probability values were inferred by a permutation method described by Horvath et al, using up to 100,000 Monte Carlo samples.

Further details of the statistical analyses and adjustment methods for antihypertensive treatment are available in the Data Supplement.

Results

GRAPHIC Study Families

Table 1 shows the characteristics of the 996 subjects (from 250 families) included in the analyses. Mean 24-hour ambulatory SBP (SD) was 119.2 (10.8), and mean ambulatory DBP was 72.0 (7.7). There were significant correlations between the different ambulatory BP phenotypes and also between these phenotypes and clinic BP (Data Supplement Table I). A history of hypertension was reported by 136 (13.7%) subjects, of whom 63 (6.3%) were currently receiving antihypertensive treatment.

Allele Frequencies and LD

Table 2 summarizes the genomic location, allele frequency, and Hardy-Weinberg tests for the 9 WNK1 SNPs analyzed. None of the SNPs showed statistically significant deviation from Hardy-Weinberg equilibrium. Strong, pairwise LD was observed between the intragenic SNPs from intron 1 to intron 26 (Data Supplement Figure 1). The WNK4 SNP (1156666G→A) had a minor allele frequency of 11.1% and did not deviate from Hardy-Weinberg equilibrium (P=0.62).

Heritability

The estimated proportion of the BP variance attributable to additive polygenic effects (ie, the narrow-sense heritability, or \( h^2_n \)) was 63.4% (95% CI, 52.3% to 73.3%) for mean 24-hour SBP and 67.9% (95% CI, 57.5% to 77.2%) for mean 24-hour DBP.

Primary Association Analyses

Of the 9 WNK1 SNPs, 4 exhibited a significant association (\( P<0.05 \)) with mean 24-hour SBP and 5 with mean 24-hour DBP (Table 3). Furthermore, 2 of these SNPs (rs880054 in intron 10 and rs2301880 in intron 23) showed highly significant associations (\( P<0.005 \)) with mean 24-hour SBP and DBP, respectively. In addition, rs765250 in intron 1 exhibited a highly significant association (\( P<0.005 \)) with mean nighttime SBP. Under an additive genetic model, the coefficient for each SNP (Table 3) may be interpreted as the mean increase in BP (in mm Hg) associated with each additional copy of the minor allele. For example, the most common SNP rs880054 was associated with a mean reduction in 24-hour SBP of 1.37 mm Hg per copy of the C (minor) allele (95% CI,
TABLE 2. Description of the WNK1 SNPs Genotyped in the GRAPHIC Study

<table>
<thead>
<tr>
<th>WNK1 SNP*</th>
<th>Chromosome 12 Position‡</th>
<th>WNK1 Position</th>
<th>Alleles‡</th>
<th>Minor-Allele Frequency (Parents)¶</th>
<th>Hardy-Weinberg Test¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1468326</td>
<td>727762</td>
<td>5` region</td>
<td>C/A</td>
<td>0.003</td>
<td>0.88</td>
</tr>
<tr>
<td>rs2369402</td>
<td>748925</td>
<td>Intron 1</td>
<td>G/A</td>
<td>0.046</td>
<td>0.83</td>
</tr>
<tr>
<td>rs765250</td>
<td>78544</td>
<td>Intron 1</td>
<td>T/C</td>
<td>0.19</td>
<td>0.66</td>
</tr>
<tr>
<td>rs880054</td>
<td>858819</td>
<td>Intron 10</td>
<td>T/C</td>
<td>1.37</td>
<td>0.24</td>
</tr>
<tr>
<td>rs956688</td>
<td>861173</td>
<td>Exon 13</td>
<td>C/A</td>
<td>3.39</td>
<td>0.065</td>
</tr>
<tr>
<td>rs953361</td>
<td>872068</td>
<td>Intron 22</td>
<td>G/T</td>
<td>1.40</td>
<td>0.24</td>
</tr>
<tr>
<td>rs2301880</td>
<td>874098</td>
<td>Intron 23</td>
<td>G/T</td>
<td>0.55</td>
<td>0.46</td>
</tr>
<tr>
<td>rs2286028</td>
<td>885730</td>
<td>Intron 26</td>
<td>G/C</td>
<td>0.32</td>
<td>0.57</td>
</tr>
</tbody>
</table>

*dsSNP accession number.
¶Major/minor alleles shown.
§Number of subjects with genotype data available for analysis.
||Minor allele frequency and Hardy-Weinberg tests relate to the parents (parental generation).

2.45-mm Hg reduction to a 0.23-mm Hg reduction). We found no evidence of modification of the effect of any of the WNK1 SNPs on mean 24-hour SBP or DBP by sex. The WNK4 SNP (1156666G→A) was not associated with any of the BP phenotypes.

Secondary Association Analyses
Secondary analyses were carried out to examine (1) the association between WNK1 variants and clinic BP; (2) the effect of different genetic models, and (3) the impact of adjustments for additional covariates. Three WNK1 SNPs were associated (P<0.05) with clinic SBP and 5 with clinic DBP (P=0.00087 for association between rs880054 and clinic DBP; Data Supplement Table II). Significant associations were observed between WNK1 SNPs and mean 24-hour SBP and DBP under different genetic models (Data Supplement Tables III to V). The data are consistent with a codominant effect for the majority of SNPs, but a dominant effect cannot be ruled out. Significant associations between the WNK1 SNPs and mean 24-hour SBP and DBP were also evident after correcting for body mass index in addition to age and sex (Data Supplement Table VI) and also after including a range of other covariates, such as smoking, alcohol intake, history of hypercholesterolemia, and educational level (Data Supplement Table VII).

Haplotype Analyses of WNK1: Joint Linkage and Association
Fifteen haplotypes with a frequency of 1% or greater were identified, and 4 of these showed significant evidence (P<0.05) of an association in the presence of linkage with mean 24-hour SBP and/or mean 24-hour DBP (Table 4). Of these, 2 haplotypes were common: h2 ‘GTCTTCCTC’ and h4 ‘CACCCCCG’ (frequencies of 0.158 and 0.126, respectively). Haplotype h2 was associated with a significant rise in mean 24-hour DBP, whereas h4 was associated with a fall in both mean 24-hour SBP and mean 24-hour DBP. Another haplotype, h7 ‘CGCTTCCG’ (frequency, 0.040), was associated with a rise in BP across all BP phenotypes, with significant associations with 5 of the 6 ambulatory BP

TABLE 3. Estimates of the Effects of the WNK1 SNPs and WNK4 SNP on Ambulatory SBP and DBP Phenotypes

<table>
<thead>
<tr>
<th>SNP</th>
<th>Mean 24-Hour SBP</th>
<th>Mean Daytime SBP</th>
<th>Mean Nighttime SBP</th>
<th>Mean 24-Hour DBP</th>
<th>Mean Daytime DBP</th>
<th>Mean Nighttime DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNK1</td>
<td>Coefficient (95% CI)</td>
<td>P Coefficient (95% CI)</td>
<td>P Coefficient (95% CI)</td>
<td>P Coefficient (95% CI)</td>
<td>P Coefficient (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>rs1468326</td>
<td>-0.94 (-2.60 to 0.75)</td>
<td>0.32</td>
<td>-1.08 (-2.94 to 0.94)</td>
<td>0.22</td>
<td>-0.87 (-2.02 to 0.33)</td>
<td>0.15</td>
</tr>
<tr>
<td>rs2369402</td>
<td>-1.42 (-2.75 to -0.09)</td>
<td>0.037</td>
<td>-1.45 (-2.77 to -0.09)</td>
<td>0.031</td>
<td>-0.98 (-1.87 to -0.04)</td>
<td>0.036</td>
</tr>
<tr>
<td>rs765250</td>
<td>-2.49 (-2.69 to -0.24)</td>
<td>0.016</td>
<td>-1.30 (-2.44 to -0.20)</td>
<td>0.019</td>
<td>-1.24 (-1.93 to -0.58)</td>
<td>0.041</td>
</tr>
<tr>
<td>rs880054</td>
<td>-1.50 (-2.71 to 1.16)</td>
<td>0.42</td>
<td>-1.16 (-2.52 to 0.16)</td>
<td>0.095</td>
<td>-1.81 (-2.91 to -0.96)</td>
<td>0.015</td>
</tr>
<tr>
<td>rs2286007</td>
<td>-0.10 (0.04 to 0.20)</td>
<td>0.027</td>
<td>-0.11 (-0.30 to 0.09)</td>
<td>0.059</td>
<td>0.89 (0.10 to 1.67)</td>
<td>0.026</td>
</tr>
<tr>
<td>rs956688</td>
<td>1.01 (0.11 to 2.02)</td>
<td>0.072</td>
<td>0.85 (0.29 to 2.05)</td>
<td>0.14</td>
<td>1.12 (0.09 to 2.18)</td>
<td>0.059</td>
</tr>
<tr>
<td>rs2301880</td>
<td>-1.78 (-3.00 to -0.59)</td>
<td>0.0045</td>
<td>-1.12 (-3.04 to -0.89)</td>
<td>0.017</td>
<td>-2.0 (-3.24 to -0.81)</td>
<td>0.002</td>
</tr>
<tr>
<td>rs2286028</td>
<td>0.99 (-0.40 to 2.38)</td>
<td>0.17</td>
<td>1.12 (-0.45 to 2.68)</td>
<td>0.16</td>
<td>0.93 (-0.53 to 2.38)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

All analyses took full account of familial relationships and were adjusted for age, age², and sex as covariates. The coefficients are shown under an additive genetic model and may be interpreted as a per-allele effect. Significant results (P<0.05) are shown in boldface type.
Although the GLMM-based approach is robust to stratification, the results are supported by the results of those based on the individual SNPs. The haplotype analyses provide further reassurance that these findings were not solely attributable to population substructure. Further, although genetic association studies should not be subject to confounding by lifestyle factors, significant associations were still noted between WNK1 SNPs and mean 24-hour SBP and DBP after correction for a range of covariates. Although we did not apply a Bonferroni correction, our findings cannot simply be explained by multiple testing. We tested a limited number of WNK1 SNPs in strong LD and found at least 1 significant association for each common SNP. Furthermore, 2 associations with mean 24-hour BP phenotypes and 2 associations with nighttime BP phenotypes were highly significant (P<0.005), and even the low-power global test supported significant linkage and association of the WNK1 haplotypes with mean 24-hour DBP (P=0.011).

The GRAPHIC study adopted a design and analytic strategy that optimized the ability to detect genetic determinants of BP. First, we studied BP as a continuous trait. This is a powerful approach, particularly when measurement error is minimized. We therefore used ambulatory BP monitoring, the most precise noninvasive measure of usual BP that is available. Second, through participating family practices, we generated a study population that was representative of the general population, which itself showed significant associations with BP. Although family-based association tests for haplotypes were used for the analysis, implemented with the HBAT extension of the FBAT toolkit. Significant results (P<0.05) are shown in boldface type.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Mean 24-Hour SBP</th>
<th>Mean Daytime SBP</th>
<th>Mean Nighttime SBP</th>
<th>Mean 24-Hour DBP</th>
<th>Mean Daytime DBP</th>
<th>Mean Nighttime DBP</th>
</tr>
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<tbody>
<tr>
<td>Alleles</td>
<td>Z</td>
<td>P</td>
<td>Z</td>
<td>P</td>
<td>Z</td>
<td>P</td>
</tr>
<tr>
<td>CGTCTCG h1 0.175 120 0.361 0.73 0.240 0.81 0.494 0.62 0.726 0.47 0.911 0.35 0.333 0.73</td>
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<tr>
<td>CGTCTTC h2 0.158 106 −0.512 0.61 −0.473 0.64 −0.498 0.62 2.291 0.021 1.966 0.052 1.962 0.048</td>
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<tr>
<td>CGTCCGG h3 0.139 98 0.206 0.84 0.233 0.81 0.049 0.96 −1.403 0.16 −1.207 0.23 −1.555 0.12</td>
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<tr>
<td>CACCCCCG h4 0.126 84 −2.264 0.025 −2.230 0.023 −1.854 0.064 −2.159 0.032 −2.142 0.032 −2.152 0.014</td>
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<tr>
<td>GTCTAGC h5 0.126 80 −0.663 0.51 −0.843 0.40 −0.271 0.78 −1.287 0.20 −1.146 0.26 −1.103 0.27</td>
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<tr>
<td>CACTCCG h6 0.074 58 1.847 0.058 1.980 0.047 1.391 0.17 1.210 0.22 1.030 0.30 1.245 0.21</td>
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<tr>
<td>CGGCCCCG h7 0.040 32 2.248 0.022 2.450 0.014 1.616 0.11 2.707 0.0053 2.576 0.0082 2.357 0.014</td>
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<tr>
<td>AGGCCCCG h8 0.024 21 −1.592 0.11 −1.010 0.32 −1.646 0.095 −0.471 0.64 −0.086 0.94 −0.437 0.66</td>
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<tr>
<td>CGGCCCCG h9 0.023 23 −1.749 0.083 −1.161 0.25 −2.099 0.038 −2.117 0.032 −1.683 0.091 −1.961 0.047</td>
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<tr>
<td>AGCCTGG h10 0.016 15 0.352 0.73 0.450 0.65 0.089 0.91 −0.148 0.89 −0.037 0.97 −0.246 0.82</td>
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<tr>
<td>CGTCTGG h11 0.016 16 0.432 0.67 0.422 0.68 0.488 0.63 −1.381 0.17 −1.408 0.16 −0.876 0.39</td>
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<tr>
<td>AGCTCTC h12 0.016 16 0.055 0.95 −0.402 0.70 0.615 0.55 −1.213 0.23 −1.46 0.15 −0.283 0.78</td>
<td></td>
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<tr>
<td>AGTCTGG h13 0.014 14 0.438 0.68 0.393 0.70 0.386 0.69 −0.470 0.66 −0.720 0.48 −0.079 0.94</td>
<td></td>
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<tr>
<td>AGCCCGG h14 0.012 10 1.701 0.077 2.038 0.034 0.712 0.51 0.675 0.53 1.286 0.22 −0.235 0.81</td>
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<tr>
<td>AACCCCCG h15 0.010 10 0.535 0.60 −0.143 0.89 0.978 0.34 0.695 0.50 0.332 0.76 0.602 0.54</td>
<td></td>
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</tr>
</tbody>
</table>

*Family-based association tests for haplotypes were used for the analysis, implemented with the HBAT extension of the FBAT toolkit. Significant results (P<0.05) are shown in boldface type.

†n Indicates number of informative families.

‡A negative Z score indicates a haplotype that reduces BP, and a positive Z score, a haplotype that increases BP.

§In addition to the haplotype-specific test, a more conservative global test of joint linkage and association across all haplotypes with at least 10 informative families is shown.

phenotypes (P=0.0053 for mean 24-hour DBP). In addition, h9 ‘CGCCCCCG’ (frequency, 0.023) was associated with a fall in BP across all phenotypes, with significant findings for 3 BP phenotypes. A multihaplotype (global) test of linkage and association was statistically significant for mean 24-hour DBP (P=0.011) but of only borderline significance for mean 24-hour SBP (P=0.058). Of interest, the 2 haplotypes with the most discordant estimated effects were h4 and h7. These haplotypes differed in the alleles of rs2369402 and rs880054, which themselves showed significant associations with BP (Table 3).

**Discussion**

We report statistically significant associations of common SNPs spanning introns 1 to 26 of the WNK1 gene with ambulatory SBP and DBP in the general population. All SNPs that had a minor-allele frequency of >0.15 showed significant association with at least 1 ambulatory BP phenotype.

Any reported genetic association should be interpreted with caution until it is replicated. However, the following evidence supports a genuine rather than a chance or artificial association between WNK1 variants and BP. First, there is a priori evidence of involvement of the gene in a monogenic form of hypertension. Second, power calculations a priori and post hoc suggest that this study was adequately powered (see Data Supplement). Third, the haplotype analyses support the results of those based on the individual SNPs. Although the GLMM-based approach is robust to stratification and admixture, the haplotype analyses provide further reassurance that these findings were not solely attributable to population substructure. Fourth, although genetic association studies should not be subject to confounding by lifestyle factors, significant associations were still noted between WNK1 SNPs and mean 24-hour SBP and DBP after correction for a range of covariates.

Although we did not apply a Bonferroni correction, our findings cannot simply be explained by multiple testing. We tested a limited number of WNK1 SNPs in strong LD and found at least 1 significant association for each common SNP. Furthermore, 2 associations with mean 24-hour BP phenotypes and 2 associations with nighttime BP phenotypes were highly significant (P<0.005), and even the low-power global test supported significant linkage and association of the WNK1 haplotypes with mean 24-hour DBP (P=0.011).

The GRAPHIC study adopted a design and analytic strategy that optimized the ability to detect genetic determinants of BP. First, we studied BP as a continuous trait. This is a powerful approach, particularly when measurement error is minimized. We therefore used ambulatory BP monitoring, the most precise noninvasive measure of usual BP that is available. Second, through participating family practices, we generated a study population that was representative of the English population in terms of BP and age-appropriate prevalence and treatment of hypertension. This sampling strategy not only allows generalizability of the findings but also avoids loss of power with adjustment for ascertainment bias. Third, we used censored normal and nonparametric
approaches to adjust for the effects of antihypertensive therapy. These methods avoid the bias and loss in power that arise from inappropriate correction for treatment effects. Fourth, the nuclear family–based design of the GRAPHIC study permitted the study of variance components and heritability and is robust to population stratification. Our estimates of heritability are consistent with other studies that have minimized the measurement error for BP.

The effects of the WNK1 variants on BP may seem modest. For example, the proportion of the additive polygenic variance in mean 24-hour SBP explained by SNP rs2301880 is \( \approx 1.4\% \) (ie, 0.9% of the total variance). However, the estimated magnitude of these effects is entirely consistent with what might be expected for a complex genetic trait. Importantly, even a 2-mm Hg-lower usual SBP is associated with an \( \approx 10\% \) fall in stroke mortality and a 7% reduction in mortality from ischemic heart disease or other vascular causes in middle age. SNP rs2301880 exhibited a minor-allele frequency of 26% and a per-allele reduction in SBP of 1.78 mm Hg, a difference of \( >3.5\) mm Hg between TT and CC homozygotes. The magnitude of the estimated effect is comparable to that seen with a modest reduction in dietary sodium. These observations highlight the potential public health importance of our findings.

No other study to date has reported an association between WNK1 variants and BP in a population-based sample. Kokubo et al found no association between WNK1 SNPs and clinic SBP or DBP in a population of 771 hypertensives and 1047 controls selected from within the Suita cohort in Japan. Recently, the BRIGHT study showed nominal evidence of an association between WNK1 SNP rs1468326 and SBP and DBP in a family study of extremely hypertensive subjects.

Only 1 common SNP has been reported in WNK4 in white European subjects. This is in intron 10 (1156666G>A), and there are conflicting reports of its association with hypertension. We observed allele frequencies of this SNP similar to those in previous studies but found no association with either ambulatory or clinic BP in our population. We have also confirmed that several other SNPs reported in WNK4 in dbSNP are not polymorphic in white Europeans and have also undertaken an SNP screening project of the promoter and functional domains of WNK4 by direct sequencing of 20 individuals with divergent WNK1 haplotypes (S.N. and P.B.M., unpublished data). No novel SNPs were found. Our results are consistent with those of Erlich et al and suggest that WNK4 does not contain common polymorphisms in white Europeans.

Our study shows the potential importance of WNK1 in BP regulation in humans. This is the first study to show that a gene causing a monogenic form of hypertension plays a significant role in BP regulation in the general population. Given the function of WNK1 in renal sodium and potassium homeostasis, it will be important to investigate whether there are interactions between WNK1 variants with modifiable environmental exposures, such as dietary salt intake. If such interactions are found, then the modification of such exposures may lead to a disproportionate effect in certain population subgroups with important health consequences. Even greater public health benefits might be realized if a more robust understanding of the biological pathways through which WNK1 exerts its effects leads to identification of an intermediate phenotype that might be amenable to modification in whole populations. In addition, if knowledge of the role of WNK1 in BP regulation were to lead to the development of antihypertensive drugs with improved efficacy or acceptability, then substantial improvements in BP control in the treated hypertensive population may be achieved. Given that thiazide diuretics cause a particularly large fall in BP in PHAII patients, pharmacogenetic studies to establish whether or not the efficacy, side effects, and acceptability of different classes of antihypertensive agents vary with polymorphisms in the WNK1 gene may become relevant if our findings are confirmed in other studies.

Limitations
We studied a relatively healthy, young to middle-aged white European population. Although BP values were broadly representative of the English general population, the generalizability of our findings to older age groups, less healthy individuals, and different ethnic groups needs to be established. Furthermore, we have not identified the causal variants in WNK1 that are responsible for the effect on BP. WNK1 is a relatively large gene, and although we assumed that the 8 tSNPs lie in a single block, it is also possible that the tSNPs could span \( >1\) haplotype block. The SNPs showing a significant association spanned almost the whole WNK1 gene (from intron 1 to intron 26), and all were intronic and unlikely to be functional. Therefore, further investigations are required to pinpoint specific regions of the WNK1 gene that harbor functional genetic variants. Finally, the mechanisms by which any causal variants in WNK1 affect BP need to be elucidated.

In summary, common variants in the WNK1 gene contribute to BP variation in the general population. The findings provide a basis to identify functional variants of WNK1, elucidate any interactions of these variants with dietary intake or the response to antihypertensive drugs, and determine their impact on cardiovascular morbidity and mortality.

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References


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