WNK4 Intron 10 Polymorphism Is Not Associated With Hypertension
Helen J.L. Speirs and Brian J. Morris

Hypertension. 2004;43:766-768; originally published online February 16, 2004;
doi: 10.1161/01.HYP.0000120121.43524.cd

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/43/4/766

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/
WNK4 Intron 10 Polymorphism Is Not Associated With Hypertension

Helen J.L. Speirs, Brian J. Morris

Abstract—A polymorphism in intron 10 of the serine-threonine kinase with no lysine (K) 4 gene WNK4 (G→A, base 1156666 on chromosome 17) has recently been associated with essential hypertension in a white American population. We have attempted to replicate this finding in a well characterized cohort of 184 unrelated hypertensive Australians of British extraction in which biological power was enhanced by them each having 2 hypertensive parents. Controls were 219 normotensive ethnically matched subjects whose parents were both normotensive. Genotyping was performed using the homogeneous MassEXTEND Assay. This showed a frequency of 0.10 for the minor allele in each group (P=0.88). Moreover, blood pressure, body mass index, sex, and plasma lipid levels were similar across genotypes. In conclusion, our study provides no support for an association of the intron 10 variant of WNK4 with essential hypertension in the Anglo-Australian population studied. (Hypertension. 2004;43:766-768.)

Key Words: kinase ■ hypertension, essential ■ genetics ■ polymorphism ■ sodium transport

A locus for essential hypertension has been reported on chromosome 17 in several chromosomal microsatellite scans,1–3 as had been noted for the equivalent region in the genome of genetically hypertensive rats.4,5 Recently, interest has focused on WNK4 (chromosome 17q21.2), which encodes a serine-threonine kinase with no lysine (K), but instead has a cysteine at a key position in the active site. Such interest was sparked by the finding that mutations in WNK4 are responsible for a monogenic form of hypertension pseudohypoaldosteronism type II (PHAII; Online Mendelian Inheritance in Man no. 145260),6 an autosomal dominant disorder, which is characterized by severe hypertension with hyperkalemia and metabolic acidosis. WNK4 is expressed almost exclusively in the kidney, where it localizes to the distal convoluted tubule and the cortical collecting duct, more specifically to the intercellular junctions in the distal convoluted tubule and in both the cytoplasm and intercellular junctions in the cortical collecting duct.6 Although essential hypertension clearly differs from rare monogenetic hypertensive disorders, genes for the latter have previously been implicated in Liddle’s syndrome7,8 (a severe mutation of which was originally implicated in the rare hypertensive condition glucocorticoid-remediable aldosteronism9,10) and also of the epithelial sodium channel11–13 (severe mutations of which have been implicated in Liddle’s syndrome10,13,14).

A SNP in WNK4 (G→A, intron 10; bp 1156666) was reported recently in Hypertension to be associated with essential hypertension in white Americans.15 A finding of such potential importance calls for urgent confirmation in other settings. We therefore tested this SNP in case-control groups with high biological power as a result of them having parents who both had the same blood pressure (BP) status as the subject. These cohorts have been studied extensively and have been shown to be capable of demonstrating genetic associations with hypertension, should these be present.8,16–18

Methods

Subjects

There were 184 unrelated essential hypertension patients and 219 unrelated normotensive controls. All subjects were volunteers from Sydney and surrounding areas and were of British ancestry. Ascertainment details have been described previously.8,16–19 To be included, subjects had to be free of diabetes, heart disease, renal disease, or secondary causes of hypertension. Hypertension was defined as having a systolic/diastolic BP of ≥140/90 mm Hg on 3 separate occasions before treatment. Normotensive subjects had a BP <130/90 mm Hg. Only hypertensives who were the offspring of parents who both had hypertension and normotensives whose parents were both normotensive after age 50 were included. Hypertensives with 2 affected parents represent 10% of hypertensive patients,19 ie, the hypertensives selected had been drawn from a population of 1840 hypertensives. By using such selection criteria, we have increased the power of this type of study to reveal any genetic contribution to the trait and were able to simultaneously reduce the sample size required. For example, for a polymorphism with a minor allele frequency of 0.20 that contributes to hypertension with a disease odds ratio of 1.5, the n value required for 80% power to detect an existing association could be reduced by >75% (unpublished calculations). The study had ethical approval and all subjects gave informed consent.

Genotyping

DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen). The genomic sequence for WNK4 was obtained

Received September 30, 2003; first decision October 22, 2003; revision accepted January 20, 2004.

From the Basic & Clinical Genomics Laboratory, School of Medical Sciences and Institute of Biomedical Research, University of Sydney, Australia. Correspondence to Brian J. Morris, Basic & Clinical Genomics Laboratory, School of Medical Sciences and Institute of Biomedical Research, Building Fl3, University of Sydney, NSW 2006, Australia. E-mail brianm@physiol.usyd.edu.au

Hypertension is available at http://www.hypertensionaha.org DOI: 10.1161/01.HYP.0000120121.43524.ed

Downloaded from http://hyper.ahajournals.org/ by guest on February 27, 2014
from the Ensembl Human Genome browser (http://www.ensembl.org/Homo_Sapiens). The G→A polymorphism in intron 10 of the WNK4 gene \(^{13}\) was detected using the homogeneous MassEXTEND Assay (Sequenom, http://www.sequenom.com). The MassEXTEND assay is based on primer extension whereby allele-specific polymerase chain reaction products are produced. Each primer extension product has a unique molecular mass, which allows genotyping by MALDI-TOF mass spectrometry. Genotyping was performed by the Australian Genome Research Facility.

### Statistical Analysis

Total observed alleles on all chromosomes were calculated from genotype data. \(\chi^2\) Analysis was used to test for differences in allele and genotype frequencies between hypertensive patients and normotensive controls. Comparison of demographic parameters across genotypes was by one-way ANOVA. Each was conducted using StatView (Abacus Concepts). Linkage disequilibrium was tested in the largest group, the normotensives, by the method of Hill.\(^{20}\)

### Results

The characteristics of normotensive control subjects and hypertensive patients are shown in Table 1. Genotype frequencies did not deviate from Hardy-Weinberg equilibrium. Genotype and allele frequencies in the hypertensive group were similar to those in the normotensive group (Table 2) (for alleles, OR=1.0; 95% CI: 0.6 to 1.5). For a relative risk of hypertension of 1.6, our study had 95% power to detect a significant association (\(P<0.05\)) of the WNK4 variant with hypertension. Comparison across genotypes for the parameters in Table 1 also showed no significant differences. In the case of pretreatment systolic BP, values were 172±25, 178±26, and 191±30 mm Hg (n=120, 19, and 2, respectively; \(P=0.37\)), and for diastolic BP were 106±15, 108±14, and 110 mm Hg (n=118, 19, and 1, respectively; \(P=0.84\)).

The intron 10 variant was not in linkage disequilibrium with another polymorphism in intron 10 \(^1\) (\(D'=0.037; \ P=0.05\)), and haplotype analysis of these 2 variants between the hypertensive and normotensive groups failed to show any association with hypertension (data not shown).

### Discussion

Our data presented here argue strongly against the hypertension association for WNK4 reported recently by Erlich et al.\(^{15}\) applying to our hypertensive population, and raises the question that, at best, the previous association finding may not be generally applicable. The present finding adds, moreover, to our previous negative finding for another variant in intron 10 of WNK4.\(^{21}\) Erlich et al noted the association with hypertension in white, but not in black, hypertensives.\(^{15}\) Whereas we found that the frequency of the minor allele was 0.10 in each group, they found frequencies of 0.13 in 165 hypertensives and 0.07 in 91 normotensives. The significance of this difference was marginal (\(P=0.04\)). Moreover, they tested 8 polymorphisms, and given such multiple comparisons, their finding could represent a false-positive. Furthermore, their white study groups appeared to contain greater genetic heterogeneity than ours, which were restricted to white subjects of Anglo-Celtic extraction. In addition, the selection of our hypertensive population on the basis of a strong family history of hypertension meant that our cohort of patients, because of inherent biological power, had an increased likelihood of demonstrating a genetic contribution to hypertension. Thus, if an association of the WNK4 variant with hypertension existed in our population, our study should have been able to detect it.

Comparative analysis has found the entire coding sequence to be identical for WNK4 in stroke-prone spontaneously hypertensive and normotensive Wistar-Kyoto rats, and thus failed to support a role of WNK4 in polygenic hypertension.\(^{23}\) Moreover, no differences in WNK4 gene expression were observed in the distal nephron of kidneys from each strain and a secondary effect of BP on transcriptional regulation was excluded.\(^{23}\)

Although a linkage peak for hypertension in the WNK4 region has been reported in several studies,\(^{1–3}\) many genome scans have failed to see either a hypertension or a BP linkage peak in this region.\(^{24,25}\) It has been pointed out recently\(^{26}\) that the greatest statistical support for the locus on chromosome 17 was in a study by Julier et al\(^{1}\) of groups recruited on the basis of diabetes. In the study by Baime et al, the rapid decay in linkage seen over a small chromosomal area of <1 cm\(^2\) is consistent with inaccurate marker placement and hence inter-marker distance or, less

### Table 1. Characteristics of Normotensive and Hypertensive Study Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensives</th>
<th>Hypertensives</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N)</td>
<td>219</td>
<td>184</td>
<td></td>
</tr>
<tr>
<td>Male:Female (%)</td>
<td>58:42</td>
<td>39:61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (y)</td>
<td>44±12</td>
<td>52±12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>25±4</td>
<td>27±5</td>
<td>&lt;0.0003</td>
</tr>
<tr>
<td>Systolic BP pretreatment (mm Hg)</td>
<td>119±10</td>
<td>173±25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP pretreatment (mm Hg)</td>
<td>72±8</td>
<td>106±15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.8±0.07</td>
<td>4.9±0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3±0.05</td>
<td>1.9±0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.3±0.03</td>
<td>1.1±0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol (mol/L)</td>
<td>3.7±0.07</td>
<td>3.7±0.1</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Values shown are mean ±SD, except for plasma parameters, which are ±SE. BMI indicates body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

### Table 2. Genotype and Allele Frequencies of the 115666 G→A WNK4 Polymorphism in Normotensive and Hypertensive Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>(N)</th>
<th>(GG)</th>
<th>(GA)</th>
<th>(AA)</th>
<th>(\chi^2)</th>
<th>(P)</th>
<th>Allele Frequencies</th>
<th>(\chi^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensives</td>
<td>219</td>
<td>176</td>
<td>43</td>
<td>0</td>
<td>4.5</td>
<td>0.12</td>
<td>((0.80))</td>
<td>0.90</td>
<td>0.10</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>184</td>
<td>142</td>
<td>29</td>
<td>3</td>
<td>333</td>
<td>35</td>
<td>((0.82))</td>
<td>0.90</td>
<td>0.10</td>
</tr>
</tbody>
</table>
likely, to allele mis-specification. The study having the greatest power and best study design is that of Levy et al., and those studies that failed to find a chromosome 17 linkage peak might have been underpowered or comprised subjects with phenotypic differences, such as obesity, meaning that their hypertension may have a different genetic basis. Although the largest genome scan performed to date yielded a negative result for chromosome 17, the possibility remains of a minor contributor locus here or one that manifests only in, for example, diabetic hypertension.

In conclusion, the present investigation offers no support for a previous association of an intron 10 polymorphism of WNK4 and essential hypertension. Our finding further lessens, but does not completely eliminate, the possibility that WNK4 could play a part in the cause of essential hypertension. Because it is unclear what functional significance this intron polymorphism would have, other polymorphisms in the coding or regulatory regions of WNK4 should perhaps be tested before ruling out completely the involvement of WNK4 in the cause of essential hypertension.

**Perspectives**

Mutations in WNK4 have been identified as causing the rare monogenic form of hypertension PHAII. The conjecture that it is possible that mutations in WNK4, which cause more subtle changes than those observed in PHAII, may contribute to polygenic hypertension is attractive, particularly because WNK4 is located in a region that demonstrates a quantitative trait locus for hypertension in humans and rats. The results of our association studies fail to support a role for WNK4 in essential hypertension in our Anglo-Australian population. Future work should be directed at other genes under the chromosome 17 linkage peak, but only in populations in which there is convincing evidence that a linkage peak truly exists in this region. The possibility that a true contributory locus to hypertension might only exist in diabetic subjects, whose blood pressure elevation might have a different cause than classic essential hypertension, should be borne in mind. Whether this could involve WNK4, or whether a completely different gene is involved, requires extensive further investigation. More work is also needed to see whether involvement of WNK4 could be restricted to a particular population, ethnic, or racial group.

**Acknowledgments**

This study was supported by a grant from National Health and Medical Research Council of Australia. We thank Judith O’Neill for help in the collection of patient samples and Adam V. Benjafeld for assistance with some of the statistical analyses.

**References**


Downloaded from http://hyper.ahajournals.org/ by guest on February 27, 2014