Association of NEDD4L Ubiquitin Ligase With Essential Hypertension

Christopher J. Russo, Efthymia Melista, Jing Cui, Anita L. DeStefano, George L. Bakris, Athanasios J. Manolis, Haralambos Gavras, Clinton T. Baldwin

Abstract—NEDD4L is a ubiquitin ligase that controls cell surface expression of kidney epithelial Na\(^+\) channels by ubiquitin-mediated endocytosis and lysosome targeting. Thus, it is a significant determinant of Na\(^+\) reabsorption in the distal nephron. The NEDD4L gene is located on human chromosome 18q21 within several blood pressure quantitative trait loci, including those for familial orthostatic hypotension, essential hypertension, pulse pressure, and systolic blood pressure response to postural challenge. Because of the importance of NEDD4L to Na\(^+\) balance, many of these studies have proposed that mutations in NEDD4L may be responsible for these blood pressure phenotypes. To test this hypothesis, we fine-mapped the NEDD4L region in 2 families with orthostatic hypotension, which we previously reported to be linked to human chromosome 18q21 but failed to implicate NEDD4L in these families. We also typed multiple NEDD4L single-nucleotide polymorphisms (SNPs) in a collection of US whites, Greek whites, and African-Americans individuals with essential hypertension. A significant association between several SNPs and hypertension was observed in all 3 populations. One of the SNPs associated in African Americans is known to result in premature truncation of the NEDD4L protein. Thus, genetic variation in NEDD4L may play a role in the development or progression of some forms of abnormal blood pressure. (Hypertension. 2005;46:488-491.)

Key Words: gene expression ▪ blood pressure ▪ hypertension, essential ▪ hypotension ▪ renal circulation ▪ sodium channels

Blood pressure regulation is a multifactorial system resulting from the complex interaction of integrated autonomic, brain, and kidney mechanisms.\(^1,2\) Elevated blood pressure is a major risk factor for stroke, myocardial infarction, and kidney failure;\(^3-5\) it is affected to a degree that >600,000,000 people worldwide, and contributes to nearly 8,000,000 deaths annually.\(^6\) A number of mendelian disorders in which abnormal blood pressure is one of several clinical findings have been identified. Frequently, these disorders (Liddle syndrome, pseudohypoaldosteronism, etc) result from a mutation in a gene important in sodium reabsorption by the kidney.\(^7\) However, it has not yet been shown that mutations in these same genes are important in common forms of abnormal blood pressure.

We previously reported linkage of a mendelian form of orthostatic hypotension (Streeter type, OMIM 143850) to human chromosome 18q21.\(^8,9\) Several candidate genes are located within this chromosomal region, including NEDD4L, which encodes a ubiquitin ligase important for downregulation of kidney epithelial Na\(^+\) channels.\(^10,11\) However, in our follow-up studies reported herein, we failed to implicate NEDD4L as the causative gene of orthostatic hypotension in these families.

Subsequent to our localization of the orthostatic hypotension locus, several human quantitative trait loci (QTLs) for abnormal blood pressure have been described in the same region, including essential hypertension,\(^12-14\) systolic and diastolic blood pressure,\(^15-18\) and postural change in systolic blood pressure.\(^19\) NEDD4L is also an important candidate gene in these blood pressure disorders, and in this study, we report a significant association between the NEDD4L single-nucleotide polymorphism (SNP) rs4149601 in a population of African Americans with essential hypertension. This SNP, located in the last nucleotide of exon 1m, leads to systematic use of an alternative splice site and generation of transcripts encoding a nonfunctional protein.\(^20\) Association with hypertension in US whites and Greek whites was observed for other SNPs in the NEDD4L gene, thus confirming our finding of an association between NEDD4L and essential hypertension.

Methods

Three families with autosomal-dominant orthostatic hypotension were described previously.\(^8,9\) Whites with essential hypertension

Received May 19, 2005; first decision June 13, 2005; revision accepted July 12, 2005.
From the Center for Human Genetics (C.J.R., E.M., C.T.B.) and the Department of Medicine (E.M., J.C., H.G., C.T.B.), Boston University School of Medicine, Boston, Mass; the Department of Biostatistics (A.L.D.), Boston University School of Public Health, Boston, Mass; the Departments of Preventive Medicine and Internal Medicine (G.L.B.), Rush-Presbyterian-St. Luke’s Medical Center, Chicago, Ill; and the Cardiology Division (A.J.M.), Tzanion Hospital, Piraeus, Greece.
Correspondence to Clinton T. Baldwin, PhD, Boston University School of Medicine, Center for Human Genetics, 715 Albany St, W408, Boston, MA 02118, E-mail: cbaldwin@bu.edu
© 2005 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000178594.63193.e0
were recruited from hypertension clinics at Boston Medical Center, Boston, Mass, and the Tzlanion Hospital, Piraeus, Greece, and have been described elsewhere.21 African-American hypertensives were recruited from the hypertension clinics at Boston Medical Center. Affected individuals either had blood pressures >140 mm Hg or were receiving antihypertensive medication. Ethnically matched normotensive subjects were recruited from the same populations from which the hypertensive individuals were ascertained and were age 55 or older, not using antihypertensive medication, and had blood pressures <130/80 mm Hg. The Protection of Human Subject Committees at all institutions approved this study, and all subjects provided informed consent.

DNA sequencing was performed by polymerase chain reaction (PCR) amplification of the target exon, purification of the product (AMPure solid-phase reversible immobilization, Agencourt), and subjecting it to a sequencing reaction (BigDye Terminator v.3.1 chemistry, Applied Biosystems) with a third primer to prime the sequencing reaction. Sequencing products were purified and applied to an ABI 3100 genetic analyzer prepared with a POP-4 capillary gel matrix.

SNP genotyping was performed with Assays-on-Demand, Assays-by-Design, or ABI SNPlex (Applied Biosystems). Genotyping products were analyzed either in an ABI Prism 7900 sequence detection system or an ABI 3730 DNA analyzer. Microsatellite marker genotyping was performed by PCR amplification of the repeat region with a FAM dye-labeled PCR primer. The product was analyzed in an ABI 3100 capillary electrophoresis instrument.

For the SNP genotyping data, Hardy-Weinberg equilibrium was assessed within each ethnic group and by disease status by a $\chi^2$ test and genotype frequencies were compared between hypertensive and control individuals within each ethnic group by a Fisher's exact test when appropriate. SNPEM22 was used to predict haplotype frequencies for 3-SNP sliding windows for hypertensives and normotensives in each ethnic group, and haplotype frequency distributions were compared between hypertensives and normotensives.

**Results**

We previously mapped orthostatic hypotension (Streeten type, OMIM 143850) to the region between D18S858 (53.05 Mb) and D18S541 (68.33 Mb) in 2 families. The NEDD4L gene (53.86 to 54.22 Mb) is at the proximal end of the linked region between a marker (D18S1148, 57.28 Mb) that is nonrecombiant with disease and the first marker (D18S858) that is recombinant in 1 affected person9 (person 5065 in Figure 1). To either include or exclude the genomic region containing NEDD4L as a candidate region, we typed 6 SNPs (rs732796, rs734779, rs182383, rs3902163, rs3865418, and rs9953409) within NEDD4L, 6 SNPs (rs11152135, rs12961034, rs1543159, rs8086565, rs595939, rs2298712, rs3816005, rs513563, rs554192, rs1573389, rs4941382, rs4149591, rs292447, rs499661, rs472847, rs4058287, rs7228980, rs5005280, rs501370, rs533502, rs474743, rs2288774, rs10515976, rs4149589, and rs4835340) in or around NEDD4L in a collection of US whites, Greek whites, and African Americans. The linkage disequilibrium (LD) pattern among these SNPs is shown for African Americans and white Americans in Figure 2. The LD in Greek whites was similar to that seen in American whites (not shown). In African Americans, 7 SNPs (rs4149601, rs11152135, rs12961034, rs1543159, rs8086565, rs595939, and rs2298712) distal to NEDD4L, and a microsatellite marker (D18S1155) at 55.21 Mb in the 2 families with orthostatic hypotension. The results indicated that person 5065 re-

mained recombinant between all markers tested and the presence of disease, suggesting that the NEDD4L gene is outside the candidate region on chromosome 18.

We further examined NEDD4L in the families with orthostatic hypotension by sequencing multiple affected and unaffected individuals for >90% of the exons encoding the functional domains of NEDD4L. Six SNPs, all previously reported in public databases (rs4149601, rs4149606, rs2304020, rs2288774, rs2288775, and rs4149608) were identified, none unique to only affected individuals. Thus, despite the attractiveness of NEDD4L as a candidate gene for orthostatic hypotension as suggested by others,19 both our mapping and sequencing data do not support this hypothesis. Because of the large number of blood pressure QTLs identified in this region,12-18 we typed 26 SNPs (rs4149601, rs11152135, rs12961034, rs1543159, rs8086565, rs595939, rs2298712, rs3816005, rs513563, rs554192, rs1573389, rs4941382, rs4149591, rs292447, rs499661, rs472847, rs4058287, rs7228980, rs5005280, rs501370, rs533502, rs474743, rs2288774, rs10515976, rs4149589, and rs4835340) in or around NEDD4L in a collection of US whites, Greek whites, and African Americans. The linkage disequilibrium (LD) pattern among these SNPs is shown for African Americans and white Americans in Figure 2. The LD in Greek whites was similar to that seen in American whites (not shown). In African Americans, 7 SNPs (rs10515976, rs4149591, rs4149601, rs513563, rs182383, rs7228980, and rs9953409) showed a significant allelic association or genotype association (the Table). One SNP (rs4149601) associated with hypertension is located in exon 1m20 and is known to result in abnormal splicing and nonfunctional protein. Two SNPs (rs513563 and rs3865418) were found associated with hypertension in US whites, and 2 other SNPs (rs4149589 and rs3865418) were associated with hypertension in Greek whites (the Table).

To further delineate the pattern of association, haplotype analysis was conducted with the 26 SNPs typed in the case and control groups by a 3-SNP sliding-window analysis according to the program SNPEM22 (Figure 2). Two haplotype windows in African Americans and 4 in US whites were found to be associated with hypertension. Importantly, there was considerable overlap between the SNPs showing association with hypertension, individually or as part of a haplotype, in all 3 populations (Figure 2). Thus, our findings of an
association between NEDD4L and hypertension are confirmed in multiple populations.

Discussion

We previously mapped orthostatic hypotension (Streeten type) to human chromosome 18q21 in several families.\(^8,9\) Subsequently, several blood pressure QTLs have been reported in the same region,\(^12–19\) and many of these studies have suggested that the NEDD4L gene, which encodes a ubiquitin ligase involved in regulation of epithelial Na\(^+\) channels in the distal nephron, is an attractive candidate gene.\(^19\) In this study, we found no evidence to support the hypothesis that mutations in NEDD4L are responsible for orthostatic hypotension (Streeten type). However, we did find a significant association between several SNPs, including a common SNP (rs4149601) known to result in abnormal splicing, and essential hypertension in African Americans, American whites, and Greek whites. Although case-control SNP association studies may be subject to false-positive findings because of population stratification, it is not likely in this study, because we found independent evidence of association in all 3 populations studied. Evidence that this is an authentic hypertension-associated gene is further supported by the observations of numerous QTLs in this same region.\(^12–19\)

In our population of African Americans, we found that the A allele of SNP rs4149601 is associated with elevated blood pressure.

### SNPs Showing Association to Hypertension in 3 Hypertensive Populations

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Genotypic Frequency</th>
<th>Alllic Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>African Americans</td>
<td>rs10515976</td>
<td>53,965,251</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>rs4149591</td>
<td>53,966,182</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td>rs4149601</td>
<td>53,966,015</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>rs513563</td>
<td>53,025,439</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>rs513563</td>
<td>53,046,735</td>
<td>212</td>
</tr>
<tr>
<td></td>
<td>rs7228980</td>
<td>54,136,429</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>rs9953409</td>
<td>54,213,617</td>
<td>214</td>
</tr>
<tr>
<td>US whites</td>
<td>rs513563</td>
<td>54,025,439</td>
<td>181</td>
</tr>
<tr>
<td></td>
<td>rs3865418</td>
<td>54,155,144</td>
<td>205</td>
</tr>
<tr>
<td>Greek whites</td>
<td>rs4149589</td>
<td>53,966,015</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>rs3865418</td>
<td>54,155,144</td>
<td>150</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in text. SNPs and their position on chromosome 18 are indicated by public database “rs” accession numbers (http://www.ncbi.nlm.nih.gov). By convention, the major allele is indicated as “1” and the minor allele as “2”.

Nominal $P$ value <0.05.
pressure. This allele results in utilization of a cryptic splice site at the end of exon 1m, leading to the inclusion of a premature stop codon. The more common G allele is “leaky,” and both normal and abnormal transcripts are produced. Thus, the A allele of SNP rs4149601 would be predicted to reduce the ubiquitination and degradation of epithelial Na⁺ channels. A higher density of epithelial Na⁺ channels or a longer residence time on the cell surface would lead to increased transepithelial Na⁺ transport, a shift to positive Na⁺ balance, and initiation of mechanisms to raise blood pressure. However, not all transcripts contain this alternatively spliced exon; thus, only a portion of the NEDD4L transcripts would be predicted to be affected by this SNP.

Our results fail to implicate NEDD4L as the causative gene of orthostatic hypotension (Streeten type); however, this finding is dependent on a single recombination event in 1 person, and the result should be considered tentative. It is possible that several genes in the candidate region on chromosome 18 contribute to blood pressure regulation. This hypothesis is supported by the fact that not all of the blood pressure QTLs reported in the region overlap each other. Another candidate gene in the linked region is the melanocortin receptor 4, which has been implicated in the regulation of food intake, body weight, and blood pressure.

**Perspectives**

Finding the causative gene of abnormal blood pressure is of great significance and will lead to new diagnostic tests that identify individuals who are at risk for developing hypertension later in life. It provides novel targets for pharmacologic agents that can lower blood pressure in hypertensive patients to normotensive levels. This remains a significant clinical challenge, because it has been estimated that only half of hypertensive patients receive pharmacologic treatment, and of those so treated, only half achieve blood pressure control. Furthermore, finding a variation affecting Na⁺ handling may help to define a genetic profile that correlates with salt sensitivity and helps provide a valuable diagnostic tool for identifying salt-sensitive individuals.

**Acknowledgment**

This study was supported by grant P50-HL55001 from the National Institutes of Health–National Heart, Lung, and Blood Institute, Bethesda, Md.

**References**


Association of NEDD4L Ubiquitin Ligase With Essential Hypertension
Christopher J. Russo, Efthymia Melista, Jing Cui, Anita L. DeStefano, George L. Bakris,
Athanassios J. Manolis, Haralambos Gavras and Clinton T. Baldwin

Hypertension. 2005;46:488-491; originally published online August 15, 2005;
doi: 10.1161/01.HYP.0000178594.63193.c0
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 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/46/3/488

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/