Linkage of Essential Hypertension to Chromosome 18q

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Abstract—We performed a genomewide scan with 904 microsatellite markers using 120 extended Icelandic families with 490 hypertensive patients. The families were identified by cross-matching a list of hypertensive patients from the Hypertension Clinic of the University Hospital (Landspitalinn) in Iceland with a genealogy database of the entire Icelandic nation. After adding 5 markers, we found linkage to chromosome 18q with an allele-sharing LOD score of 4.60 ($P = 2.1 \times 10^{-6}$). These results provide evidence for a novel susceptibility gene for essential hypertension on chromosome 18q and show that it is possible to study the genetics of essential hypertension without stratifying by subphenotypes.

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Key Words: genetics ■ population ■ hypertension, essential

Systemic blood pressure can be described in simple terms as a product of cardiac output and systemic vascular resistance, but the control of these 2 factors is inherently complex and their regulation may be influenced by the interaction between genetic and environmental factors. Further complicating the genetic analysis of essential hypertension are several associated confounding phenotypes, including obesity, insulin resistance, and hyperlipidemia, each of which may have its own genetic component. The genetic factors contributing to the common forms of essential hypertension are unknown.1,2 Mutations in 17 genes have been shown to alter blood pressure. In 8 of these genes, the mutations produce a large increase in blood pressure resulting in rare Mendelian forms of hypertension; in the other 9, the mutations cause Mendelian forms of hypotension in humans.1 These genes all affect blood pressure through sodium reabsorption by the kidney. However, the known mutations account for only 1 in 200 cases of human hypertension. Several studies have looked for linkage and/or association of these and other candidate genes to essential hypertension, and most do not appear to contribute to essential hypertension. Of these, only the contributions of 2 have held up in multiple studies: the angiotensinogen gene on chromosome 1q42 and the α-adducin gene on chromosome 4p16.3.3

There have been several genomewide linkage scans of familial blood pressure distribution/variation4-10 and for essential hypertension.11-13 Here, we report significant linkage to chromosome 18q in our genomewide linkage study of 490 Icelandic hypertensive patients in 120 families.

Methods

Subjects

The Icelandic population is currently around 290 000 individuals. Icelanders lived in relative isolation, with very limited immigration, until the beginning of the last century. This is supported by historical and scientific evidence, both of which indicate current Icelanders to be of mixed Scandinavian and Gaelic origin, with estimates of the founding population ranging from 8000 to 20 000.14,15 The population size was limited to fewer than 70 000 individuals for most of the time since the settlement in 870 to 930 A.D. by Norse Vikings and their slaves from the British Isles.16 Because of famine and volcanic eruptions, the population went through two bottlenecks, reducing its size twice down to less than 30 000. Hypertension among Icelanders has been shown to have the same incidence as in other western societies.

The original list of hypertensive patients contained the names of 5342 individuals who had attended the ambulatory hypertension clinic at the University Hospital (Landspitalinn) of Iceland and/or had been given the diagnosis on discharge from the hospital. The entire list was run through a comprehensive genealogy database on Icelanders established at deCODE Genetics (Reykjavik, Iceland) to identify families where patients were related to one another at 6 meioses or less (eg, 6 meioses separate second cousins). The families with the largest number of patients were invited to participate. The affection status of patients was verified by confirming that they were taking antihypertensive medications as a treatment for hypertension.

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values on treatment. In the hypertension clinic at the University Hospital of Iceland, decisions about starting patients on antihypertensive medications follow the common medical practice of high blood pressure treatment. Thus, individuals with any known secondary cause for hypertension had been excluded by criteria that include normal creatinine and serum electrolyte values. For confirmation, all participants were asked by questionnaire if they had been diagnosed with elevated blood pressure and if they were being treated. The names of the drugs were recorded and confirmed to be antihypertension medications. Blood was also collected from consenting close relatives of the index cases to increase the information available for the linkage analysis. Other characteristics of the patients are given in Table 1.

This study was approved by the Data Protection Commission of Iceland and the National Bioethics Committee of Iceland. Informed consent was obtained from all patients and their relatives whose DNA samples were used in the linkage scan.

Pedigrees
A comprehensive genealogy database established at deCODE Genetics (Reykjavik, Iceland) was used to cluster the patients in pedigrees. The Data Protection Commission of Iceland reversibly encrypted each version of the computerized genealogy database before sending it to the laboratory. We used the patient list with encrypted personal identifiers as input and recursive algorithms to find all ancestors in the database who are related to any member on the input list within a given number of previous generations. The cluster function then searches for ancestors who are common to any 2 or more members of the input list. The genealogy database, together with a list of hypertensive patients, was used to identify families for linkage. In the genomewide scan, we included those hypertensive patients who were related at 6 meiotic events or fewer of another hypertensive patient.

Genotyping
The DNA samples were genotyped using 904 fluorescently labeled primer sets as previously described.

Statistical Analyses
A genomewide scan was performed using a framework map. The marker order and positions for the framework mapping set were obtained from the Marshfield genetic map (http://research.marshfieldclinic.org/genetics), except for a 3-marker putative inversion on chromosome 8. We analyzed the data using the Allegro program (deCODE Genetics) and determined statistical significance by applying affecteds-only, allele-sharing methods (which do not specify any particular inheritance model). The Allegro program, a linkage program, calculates logarithm of odds (LOD) scores based on multipoint calculations. Our baseline linkage analysis, as previously described, uses the S_pairs scoring function, the exponential allele-sharing model, and a family-weighting scheme that is halfway on the log scale between weighting each affected pair equally and weighting each family equally.

Results
We cross-matched a list of 5342 hypertension patients from the Hypertension Clinic of the University Hospital of Iceland with the genealogy database. In this study we included patients who were related at 6 or fewer meiotic events with other patients with hypertension (6 meiotic events separate second cousins). These were 490 essential hypertension patients in 120 families. Thirty-two families had at least 6 affected members each, with the largest family containing 10 affected members. The average meiotic distance between relative pairs (including parent/offspring pairs) is just over 3. The degree of relationships among the affected relative pairs is summarized in Table 2. The patients and 323 of their normotensive first-degree relatives were genotyped using over 900 microsatellite markers in a genomewide linkage scan. We analyzed the data and determined statistical significance by applying affecteds-only, allele-sharing methods (which do not specify any particular inheritance model).

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**Table 1. Demographic Information on the Affected Individuals**

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Gender Distribution</th>
<th>Average Reported Age at Diagnosis*</th>
<th>Average Body Mass Index</th>
<th>Average Reported BP at Diagnosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>95–100</td>
<td>1 0 1</td>
<td>80 (1/1)</td>
<td>29.0</td>
<td>(0/1)</td>
</tr>
<tr>
<td>90–95</td>
<td>1 0 1</td>
<td>80 (1/1)</td>
<td>22.7</td>
<td>(0/1)</td>
</tr>
<tr>
<td>85–90</td>
<td>6 0 6</td>
<td>54 (5/6)</td>
<td>25.4</td>
<td>200/105 (1/6)</td>
</tr>
<tr>
<td>80–85</td>
<td>11 3 8</td>
<td>59 (10/11)</td>
<td>25.7</td>
<td>(0/11)</td>
</tr>
<tr>
<td>75–80</td>
<td>38 14 24</td>
<td>51 (32/38)</td>
<td>27.5</td>
<td>188/103 (9/38)</td>
</tr>
<tr>
<td>70–75</td>
<td>72 30 42</td>
<td>54 (61/72)</td>
<td>26.4</td>
<td>191/105 (24/72)</td>
</tr>
<tr>
<td>65–70</td>
<td>80 39 41</td>
<td>47 (67/80)</td>
<td>28.1</td>
<td>185/103 (27/80)</td>
</tr>
<tr>
<td>60–65</td>
<td>56 20 36</td>
<td>44 (53/56)</td>
<td>29.1</td>
<td>191/108 (21/56)</td>
</tr>
<tr>
<td>55–60</td>
<td>63 36 27</td>
<td>44 (55/63)</td>
<td>28.3</td>
<td>175/110 (27/63)</td>
</tr>
<tr>
<td>50–55</td>
<td>52 24 28</td>
<td>28 (45/52)</td>
<td>29.3</td>
<td>178/111 (24/52)</td>
</tr>
<tr>
<td>45–50</td>
<td>43 18 25</td>
<td>38 (33/43)</td>
<td>27.3</td>
<td>184/113 (25/43)</td>
</tr>
<tr>
<td>40–45</td>
<td>34 14 20</td>
<td>33 (25/34)</td>
<td>28.5</td>
<td>174/111 (16/34)</td>
</tr>
<tr>
<td>35–40</td>
<td>18 3 15</td>
<td>26 (15/18)</td>
<td>28.5</td>
<td>180 /97 (8/18)</td>
</tr>
<tr>
<td>30–35</td>
<td>7 0 7</td>
<td>29 (6/7)</td>
<td>27.8</td>
<td>158/112 (4/7)</td>
</tr>
<tr>
<td>25–30</td>
<td>4 2 2</td>
<td>21 (2/4)</td>
<td>29.7</td>
<td>(0/4)</td>
</tr>
<tr>
<td>20–25</td>
<td>4 1 3</td>
<td>21 (4/4)</td>
<td>24.3</td>
<td>(0/4)</td>
</tr>
</tbody>
</table>

*Fraction in parenthesis indicates number of individuals in each age group for whom information was available per total number in age group.
Figures 1 and 2a show the LOD scores for the framework genome scan. The most prominent linkage was found to chromosome 18q with a LOD score of 3.84 \((P=1.3 \times 10^{-5})\). This locus is genomewide-significant because the single-test probability value of \(1.3 \times 10^{-5}\) corresponds to a genomewide adjusted probability value smaller than 0.05. However, the information on identity by descent-sharing in the region was 0.83, which is less complete than we prefer. To ensure that the results are a true reflection of the information contained in the material so that we can consider a linkage result significant, not only is it required that the probability value be genomewide-significant, but also that the information content in the region is at least 85%. We added 5 markers to the peak region, increasing the information content at the peak to over 0.95 and exceeding 0.90 throughout the peak region. With the additional markers, the peak region produced a LOD score of 4.60 \((P=2.1 \times 10^{-6})\) (Figure 2b). The peak is centered on marker D18S38. At this peak marker, just over half of the families, including 21 of the 32 largest families, had more

![Figure 1. Full genome scan of all affected family members using 904 microsatellite markers. The multipoint LOD score is on the vertical axis and centimorgan distance from the p-terminus of the chromosome is on the horizontal axis. Chromosome 18 is displayed in Figure 2a.](image-url)
identity by descent (IBD) sharing among affected relatives than expected for their relationship. The region determined by a drop of one in the LOD score is between markers D18S1155 and D18S814, centromeric and telomeric, respectively. The one LOD drop segment is approximately 7.5 centimorgans, and we estimate it to correspond to approximately 4.5 million bases. Four additional regions achieved a LOD score of at least 1.0. They occurred at D2S388, D11S4102, D11S937, and D17S1795, with LOD scores of 1.20, 1.43, 1.10, and 1.16, respectively.

**Discussion**

Our genomewide scan for genes that contribute to essential hypertension showed significant linkage to chromosome 18q. We included, as affected, patients taking antihypertensive medication and their first-degree relatives who were found to have moderate-to-severe essential hypertension (SBP ≥160 and/or DBP ≥95 on 2 or more different occasions). The majority of the index cases were originally found to have elevated blood pressure when participating in the Reykjavík population study of the Icelandic Heart Association, which is a longitudinal study of a large random population sample. They were subsequently referred to the hypertension clinic for further diagnosis and treatment. Our extensive computerized genealogy database on Icelanders has allowed us to identify extended families of individuals with essential hypertension, and screening these for linkage to markers with the average genomewide density of 3 to 4 cM has given highly significant results for linkage to chromosome 18q.

Several genomewide linkage scans of familial blood pressure distribution/variation have been published, most of them not attaining significance at a genomewide level.

There are, to our knowledge, 3 previous reports of genomewide scans for essential hypertension. A study based on the affected sibling-pair model using 263 affected nuclear families containing a total of 288 affecteds and 262 microsatellite markers found no significant linkage and excluded approximately 92% of the human genome. A Chinese study of 637 affected sib-pairs from 106 nuclear families gave suggestive linkage to chromosome 2q14-q23. A Finnish study based on 47 families of concordant sib pairs (mostly twins) with early-onset hypertension (onset before age 50 years) showed linkage to chromosome 3q (near Type 1 angiotensin II receptor) and suggestive linkage to 2q, 22q, and Xp.

Our genome scan shows evidence of linkage, though not significant, supporting previously linked loci on chromosomes 2, 4, 8, 11, 10, and 17 (Table 3). On chromosome 18 our genome scan identifies a locus different from previously reported loci of essential hypertension. On the other hand, it matches the location showing suggestive linkage of autosomal dominant orthostatic hypotension.

**TABLE 3. Supporting Results of Previously Linked Loci on Chromosomes 2, 11, 17, and 18**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Location</th>
<th>Study Design</th>
<th>Linkage Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2p11</td>
<td>D2S1790</td>
<td>QT Mendelian model analysis of population-based BP values.</td>
<td>3.92</td>
<td>Atwood4</td>
</tr>
<tr>
<td>2p11</td>
<td>D2S1790</td>
<td>Multipoint variance component QT analysis of population-based BP values.</td>
<td>2.2</td>
<td>Rice8</td>
</tr>
<tr>
<td>11q12</td>
<td>D11S2019</td>
<td>Extreme discordant sib-pair analysis.</td>
<td>2.07</td>
<td>Xu10</td>
</tr>
<tr>
<td>17q21</td>
<td>D17S1299</td>
<td>Multipoint QTL analysis of longitudinal BP values from the Framingham study.</td>
<td>3.8</td>
<td>Levy6</td>
</tr>
<tr>
<td>18q22</td>
<td>D18S1367</td>
<td>Autosomal dominant orthostatic hypotension.</td>
<td>3.21</td>
<td>DeStefano29</td>
</tr>
</tbody>
</table>

QT indicates quantitative trait; QTL, quantitative trait locus; BP, blood pressure.
TABLE 4. Genes Under the One-LOD-Drop Peak on Chromosome 18*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribosomal protein S26</td>
<td></td>
</tr>
<tr>
<td>Ribosomal protein, large, P0</td>
<td></td>
</tr>
<tr>
<td>CGI-39 protein; cell death-regulatory protein GRIM19</td>
<td></td>
</tr>
<tr>
<td>Phorbol-12-myristate-13-acetate-induced protein 1</td>
<td></td>
</tr>
<tr>
<td>General transcription factor IIH, polypeptide 1 (62kD subunit)</td>
<td></td>
</tr>
<tr>
<td>Ribosomal protein S3A</td>
<td></td>
</tr>
<tr>
<td>ATP synthase, H+ transporting, mitochondrial F1 complex, α subunit</td>
<td></td>
</tr>
<tr>
<td>TERA protein</td>
<td></td>
</tr>
<tr>
<td>Serologically defined colon cancer antigen 3</td>
<td></td>
</tr>
<tr>
<td>Nuclear factor (erythroid-derived 2)-like 3</td>
<td></td>
</tr>
<tr>
<td>Hypothetical protein FLJ20783</td>
<td></td>
</tr>
<tr>
<td>HSPC162 protein</td>
<td></td>
</tr>
<tr>
<td>Melanocortin 4 receptor</td>
<td></td>
</tr>
<tr>
<td>C-terminal binding protein 2</td>
<td></td>
</tr>
<tr>
<td>Ribosomal protein L30</td>
<td></td>
</tr>
<tr>
<td>Cadherin 20, type 2</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylinositol glycan, class N</td>
<td></td>
</tr>
<tr>
<td>KIAA1468 protein</td>
<td></td>
</tr>
<tr>
<td>Tumor necrosis factor receptor superfamily, member 11a, activator of NFKB</td>
<td></td>
</tr>
<tr>
<td>Ribosomal protein L17</td>
<td></td>
</tr>
<tr>
<td>Actin, β</td>
<td></td>
</tr>
<tr>
<td>Actin, α 1, skeletal muscle</td>
<td></td>
</tr>
<tr>
<td>Hypothetical protein FLJ20281</td>
<td></td>
</tr>
<tr>
<td>Hypothetical protein MGC13269</td>
<td></td>
</tr>
<tr>
<td>SCN Circadian Oscillatory Protein (SCOP)</td>
<td></td>
</tr>
<tr>
<td>Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5</td>
<td></td>
</tr>
<tr>
<td>B-cell CLL/Lymphoma 2</td>
<td></td>
</tr>
<tr>
<td>Follicular lymphoma variant translocation 1</td>
<td></td>
</tr>
<tr>
<td>Suppressor of K+ transport defect 1</td>
<td></td>
</tr>
<tr>
<td>Proteasome (prosome, macropain) 26S subunit, ATPase, 5</td>
<td></td>
</tr>
<tr>
<td>ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c</td>
<td></td>
</tr>
<tr>
<td>Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 13</td>
<td></td>
</tr>
<tr>
<td>UV-B repressed sequence, HUR 7</td>
<td></td>
</tr>
<tr>
<td>Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 3</td>
<td></td>
</tr>
<tr>
<td>Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 4</td>
<td></td>
</tr>
<tr>
<td>Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8</td>
<td></td>
</tr>
</tbody>
</table>


Perspectives

The identification of this genomewide significant locus for essential hypertension encourages us in further mapping efforts: narrowing down this locus by taking advantage of possible founder effects of the Icelandic population, looking for shared ancestral haplotypes among Icelandic hypertensive patients, and, ultimately, isolating the gene or genes involved. Replication of this result in other populations would be useful to assess the contribution of this putative gene to the more worldwide health risk. The additional smaller peaks identified in our genomewide scan provide some additional support for regions previously reported and suggest that further investigations into these regions may be warranted. Ongoing work is needed to understand the epistatic interaction of hypertension genes, as well as the interaction between the genes and environment and their impact on a variety of the relevant cofactors for this disease.

Acknowledgments

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References


A normal dominant orthostatic hypotension by DeStefano et al.29 which might be considered the opposite phenotype to hypertension and, therefore, may be allelic to hypertension. Postural systolic blood pressure response has been mapped as a quantitative trait to a nearby region on chromosome 18q.7 It is noteworthy that a linkage study done on hypertension in mice identifies a quantitative trait locus (QTL) on mouse chromosome 18q, which is syntenic to human chromosome 18q.10 Further support comes from the fact that a QTL identified in a hypertensive rat model maps to the syntenic region of chromosome 18q21.31 Listed in Table 4 are the known genes under the peak within the one-LOD-drop boundaries according to the National Center for Biotechnol-


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