Genome Scan for Blood Pressure in Dutch Dyslipidemic Families Reveals Linkage to a Locus on Chromosome 4p

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Abstract—Genes contributing to common forms of hypertension are largely unknown. A number of studies in humans and in animal models have revealed associations between insulin resistance, dyslipidemia, and elevated hypertension. To identify genes contributing to blood pressure (BP) variation associated with insulin-resistant dyslipidemia, we conducted a genome-wide scan for BP in a set of 18 Dutch families exhibiting the common lipid disorder familial combined hyperlipidemia. Our results reveal a locus on chromosome 4 that exhibits a significant lod score of 3.9 with systolic BP. In addition, this locus also appears to influence plasma free fatty acid levels (lod=2.4). After adjustment for age and gender, the lod score for systolic BP increased to 4.6, whereas the lod score for free fatty acid levels did not change. The chromosome 4 locus contains an attractive candidate gene, α-adducin, which has been associated with altered BP in animal studies and in some human populations. However, we found no evidence for an association between 2 intragenic α-adducin polymorphisms and systolic BP in this sample. We also observed suggestive evidence for linkage (lod=1.8) of diastolic BP to the lipoprotein lipase gene locus on chromosome 8p, supporting a finding previously observed in a separate insulin-resistant population. In addition, we also obtained suggestive evidence for linkage of systolic BP (lod=2.4) and plasma apolipoprotein B levels (lod=2.0) to a locus on proximal chromosome 19p. In conclusion, our genome scan results support the existence of multiple genetic factors that can influence both BP and plasma lipid parameters. (Hypertension. 2001;38:773-778.)

Key Words: genetics ■ hypertension, essential ■ dyslipidemia ■ genome scan ■ linkage analysis

Significant progress has been made in elucidating the genetic basis for certain mendelian forms of hypertension, but the genetic factors contributing to essential hypertension are largely unknown.1,2 A number of candidate gene studies and genome scans have been conducted but with varying results.3–6 The variation in these latter linkage results could be due the different racial make-up of the study populations and/or the underlying genetic heterogeneity of essential hypertension, which can clearly confound gene-finding efforts.

One strategy to reduce genetic heterogeneity is to stratify the study population by hypertension that is associated with other pathophysiologic processes, such as insulin-resistant dyslipidemia. A number of studies, those of Williams and colleagues7 in particular, have revealed associations between hyperlipidemia, insulin resistance, and elevated blood pressure (BP). These studies led to the definition of a condition similar to the metabolic syndrome termed familial dyslipidemic hypertension (FDH), involving a clustering of traits, including central obesity, lipid abnormalities, hypertension, and elevated fasting insulin levels. Several studies have demonstrated a high degree of heritability of this trait, which occurs in ~1% of the general population but in ~12% of patients with essential hypertension.8 In addition to FDH, there are other metabolic cardiovascular disorders that have overlapping features, including the atherogenic lipoprotein phenotype or the small dense LDL trait, type 2 diabetes, hyperapobetalipoproteinemia, and familial combined hyperlipidemia (FCH).9 Given these associations, we would propose that a more homogeneous hypertensive group can be selected by restricting the study population to those with hypertension associated with a specific metabolic syndrome. Thus, the potential problem of genetic heterogeneity can be reduced.

In the present study, we have utilized the common lipid disorder FCH to apply this strategy. FCH is characterized by...
insulin resistance and dyslipidemia and is present in 10% to 20% of patients with premature coronary artery disease.\textsuperscript{10–13} Individuals with FCH exhibit elevations of both plasma triglycerides and cholesterol but can frequently also have high BP and high fasting levels of plasma glucose and insulin.\textsuperscript{14} Furthermore, a recent study from our group demonstrated that one third of the FCH families in our cohort can also be classified as FDH, supporting that notion that hypertension, dyslipidemia, and insulin resistance are all components of FCH.\textsuperscript{15} In the present study, we report investigations to identify the genetic factors contributing to systolic BP (SBP) and diastolic BP (DBP) associated with insulin-resistant dyslipidemia by a genome-scan approach. Sibpair linkage analysis of the data by both 2-point and multi-point approaches revealed strong evidence for linkage of SBP to a locus on the short arm of chromosome 4. Suggestive loci are also reported, including loci exhibiting evidence for linkage to both BP and plasma lipoprotein levels.

Methods

Ascertainment of FCH Families

The 18 extended Dutch FCH families were ascertained through probands that were recruited from the Lipid Clinic of the Utrecht Academic University Hospital, as previously described.\textsuperscript{16} All subjects gave informed consent, and the study protocol was approved by the Human Investigation Review Committee of Utrecht University Hospital, The Netherlands.

SBP and DBP were measured twice with a mercury sphygmomanometer in a standardized fashion with the subject in sitting position after 10 minutes of rest. The percentage of FCH relatives and spouses with hypertension (defined as SBP $\geq$140 mm Hg and/or DBP $\geq$90 mm Hg and/or taking anti-hypertensive medication) is 44% and 28%, respectively. However, for the purposes of this study, SBP and DBP were treated as continuous quantitative traits.

Laboratory Analytical Methods

Venous blood was drawn after an overnight fast of 12 to 14 hours, and plasma was prepared by immediate centrifugation. Lipids, apolipoproteins, and measures of insulin/glucose were quantified by methods as described elsewhere.\textsuperscript{14,16} Probands or hyperlipidemic relatives who used lipid-lowering drugs were studied after their lipid-lowering treatment was withheld for 3 weeks.

Genotyping

We previously conducted a genome scan for the discrete FCH trait in these same families,\textsuperscript{17} and in the present study, the genotyping data were used to conduct a genome scan for BP. Genotypes for 2 polymorphisms in the $\alpha$-adducin gene were determined in those individuals for whom DNA and phenotype data were available (n=496). The G$\rightarrow$T substitution in exon 10 was genotyped as described previously.\textsuperscript{18} Genotyping of a C$\rightarrow$G polymorphism in exon 13 was determined by PCR amplification\textsuperscript{19} (forward primer: 5'-AAC CCC TTC ACC ACA CTC AC-3' and reverse primer: 5'-CCA CAA AGA AGC TTC CAG AG-3') followed by digestion with the restriction enzyme BstII.

Genetic Statistical Analyses

Because BP is thought to be a genetically complex trait, nonparametric linkage methods, which do not require assumptions regarding the mode of inheritance, were used.\textsuperscript{20} For the multi-point linkage analyses of the genome scan, the MAPMAKER/SIBS program was used to estimate allele sharing at and between markers.\textsuperscript{21} The test statistic for a correlation between allele sharing and squared trait difference among sibling pairs (n=322) on the proportion of alleles shared identical-by-descent. Because sibpairs in the same sibship may be nonindependent statistically, significance levels are calculated using effective degrees of freedom (edf), which reflect the number of independent sibling pairs in the analyses.\textsuperscript{22}

Association analyses of the $\alpha$-adducin polymorphisms with SBP were assessed with an ANOVA, by a measured genotype approach.\textsuperscript{24} The Stat-View program (Ver. 5.0; SAS Institute) was used to conduct these analyses, and to ensure that all observations in the data were independent, only those marrying into the pedigrees, ie, the spouse controls, were analyzed for statistical significance.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org

Results

To map the principal genes involved in BP, particularly those that may be related to dyslipidemia and insulin resistance, we performed a genome scan for SBP and DBP in FCH families. The clinical characteristics of these families are shown in Table 1. At the p-terminus of chromosome 19, suggestive evidence for linkage of SBP to a locus on the short arm of chromosome 4 was obtained and a significant lod score was observed. Furthermore, suggestive evidence for linkage of SBP and serum apoB levels was demonstrated that one third of the FCH families in our cohort can also be classified as FDH, supporting that notion that hypertension, dyslipidemia, and insulin resistance are all components of FCH.\textsuperscript{15} In the present study, we report investigations to identify the genetic factors contributing to systolic BP (SBP) and diastolic BP (DBP) associated with insulin-resistant dyslipidemia by a genome-scan approach. Sibpair linkage analysis of the data by both 2-point and multi-point approaches revealed strong evidence for linkage of SBP to a locus on the short arm of chromosome 4. Suggestive loci are also reported, including loci exhibiting evidence for linkage to both BP and plasma lipoprotein levels.

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SBP and plasma FFA levels were evaluated for linkage using the Haseman-Elston algorithm\textsuperscript{23} by regressing the squared trait difference of sibling pairs (n=322) on the proportion of alleles shared identical-by-descent. Because sibpairs in the same sibship may be nonindependent statistically, significance levels are calculated using effective degrees of freedom (edf), which reflect the number of independent sibling pairs in the analyses.\textsuperscript{22}

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observed, with peak lod scores of 2.4 and 2.0, respectively (Figure 1A). In addition, 2 suggestive loci for DBP were observed on chromosome 6 (lod = 2.5) and on chromosome 8 (lod = 1.8) (Figure 1B and 1C). Notably, the chromosome 8 locus also exhibited evidence for linkage (lod = 1.0) to waist-hip ratio and maps very close to the lipoprotein lipase (LPL) gene.

The most dramatic result from our genome scan was with a locus on the short arm of chromosome 4, which yielded highly significant linkage to SBP with a maximum lod score of 3.9 (Figure 2A). Interestingly, plasma FFA levels also mapped to this region with a peak lod score of 2.4. Re-analysis of the data excluding those individuals on antihypertensive medication resulted in a lod score of 2.6 for SBP. Of the various loci identified in the genome scan, this was the most attractive because it resulted in the highest lod score for SBP and also yielded evidence to plasma FFA levels, which are elevated in FCH and insulin-resistant individuals. Furthermore, this locus contains an excellent candidate gene, the α-chain of adducin, which has previously been associated
with BP in both rat models and certain human populations. Given the observed association between hypertension and insulin resistance, we also assessed linkage of fasting plasma insulin and glucose levels to the 4 loci identified in the genome scan. However, there was no evidence for linkage of either trait to any of these loci (data not shown).

To examine the chromosome 4 locus further, we first performed 2-point linkage analysis with 4 markers under the SBP and FFA peaks, using the SIBPAL subprogram of the SAGE package. As shown in Table 2, the results from these analyses yielded probability value that are consistent with the lod scores obtained from the multi-point analysis. We next age- and gender-adjusted the multi-point linkage analyses of SBP and FFA with the chromosome 4 markers. Importantly, the peak lod score for SBP increased to 4.6, lending further support for the contribution of this locus to BP, whereas the lod score with FFA levels did not change (Figure 2B). In addition to age and gender, SBP was also adjusted for additional covariates, such as body-mass-index and FFA, and re-analyzed. However, these results did not differ from those in which SBP was adjusted for age and gender alone (data not shown).

To assess whether α-adducin was the basis for the linkage at this locus, we genotyped 2 intragenic polymorphisms in the families. The G→T and the C→G polymorphisms are in very strong linkage disequilibrium in this Dutch sample. No significant difference was observed with mean SBP and either polymorphism in the spouse controls (Table 3A). A similar trend was also observed when all the family members were examined as 1 group (Table 3B) or when the hyperlipidemic and normolipidemic individuals were examined separately (data not shown). The lack of evidence for a contribution of the α-adducin gene to BP therefore raises the possibility that there is another gene at this locus influencing SBP in this Dutch population.

### Discussion
The strong clustering of hyperlipidemia and hypertension in FCH and other metabolic disorders with insulin-resistance

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**Table 2. Two-Point Linkage Results With Chromosome 4p Markers**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Trait</th>
<th>edf</th>
<th>2-Point P</th>
</tr>
</thead>
<tbody>
<tr>
<td>D4S2366</td>
<td>SBP</td>
<td>126</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>FFA</td>
<td>127</td>
<td>NS</td>
</tr>
<tr>
<td>D4S403</td>
<td>SBP</td>
<td>117</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>FFA</td>
<td>118</td>
<td>0.009</td>
</tr>
<tr>
<td>D4S2639</td>
<td>SBP</td>
<td>121</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>FFA</td>
<td>122</td>
<td>0.04</td>
</tr>
<tr>
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<td>SBP</td>
<td>126</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>FFA</td>
<td>128</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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![Figure 2A](http://hyper.ahajournals.org/)

**Figure 2.** A, Multi-point lod score plots for SBP and plasma FFA levels with chromosome 4 marker loci, as calculated by the MAPMAKERS/SIBS program. The arrows designate those markers under the peaks that were subsequently assessed by 2-point linkage analysis. B, Multi-point lod score plots for age- and gender-adjusted SBP and plasma FFA levels with chromosome 4 marker loci, as calculated by the MAPMAKERS/SIBS program.
Table 3. Lack of an Association Between α-Adducin Polymorphisms and SBP in the Spouse Controls and All Family Members

<table>
<thead>
<tr>
<th>Spouse controls</th>
<th>G→T (exon 10)</th>
<th>C→G (exon 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GT</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>CG</td>
</tr>
<tr>
<td>n</td>
<td>104</td>
<td>40</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>126±21</td>
<td>127±21</td>
</tr>
<tr>
<td></td>
<td>108</td>
<td>39</td>
</tr>
<tr>
<td>All family members</td>
<td>277</td>
<td>163</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>126±18</td>
<td>126±18</td>
</tr>
<tr>
<td></td>
<td>297</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>126±18</td>
<td>126±18</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

The α-adducin gene maps under the chromosome 4 lod score peak and has previously been implicated in essential hypertension among French and Italian populations but not other populations. In the present study, we did not observe evidence for an association between α-adducin and SBP in this Dutch population. This suggests that another gene in this region is affecting SBP (and plasma FFA levels) in this sample. Alternatively, our study could be limited by statistical power. Because the prevalence of FCH is about 1% and the frequency of the minor allele of each polymorphism is approximately 0.2, a larger sample size of independent FCH individuals would be required to have sufficient power to rule out whether α-adducin contributes to SBP in these dyslipidemic syndrome families.

In conclusion, we have identified loci that segregate with BP in families with insulin-resistant dyslipidemia. Although FCH individuals tend to have higher BP than unaffected subjects, they are not all hypertensive. Therefore, in this genome scan, we have identified loci that segregate with normal as well as elevated BP in the presence of FCH susceptibility genes. In addition, although the chromosome 4 locus appears to influence SBP and FFA levels in the presence of FCH susceptibility genes in this population, it does not preclude its confirmation in other samples to determine the importance of its contribution to BP variation. The strategy used in this study demonstrates the utility of searching for genes contributing to common disorders, such as hypertension, in populations that are enriched for these diseases. However, further studies are needed to confirm these findings and to understand the mechanisms by which these genes influence blood pressure.
as hypertension, by selecting a more homogeneous study sample.

Acknowledgments

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References

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