Genome-Wide Scan for Linkage to Obesity-Associated Hypertension in French Canadians


Abstract—Essential hypertension is a heterogeneous disorder that is thought to develop because of several overlapping subsets of underlying mechanisms. One such causal pathway may involve pathophysiological alterations induced by obesity. In the present study, we examined whether investigating clinically defined subtypes of hypertension, such as obesity-associated hypertension, facilitates the search for its genes. Fifty-five extended families were selected on the basis of having ≥2 siblings affected by hypertension from a geographically remote French-Canadian population. Fifteen of these families showed a high prevalence (≥70%) of obesity. Genome-wide scan using qualitative multipoint linkage analysis (GeneHunter 2.1; marker density <10 cM) was performed in the entire set of hypertensive families and the subset with high prevalence of obesity. In the scan involving all 55 families, the most significant loci (logarithm of odds [LOD] score=2.5) were identified on chromosomes 1 (D1S1597) and 11 (D11S1999). In the scan including only the subset of families with obesity-hypertension, the most significant locus (LOD score=3.1) was found on chromosome 1 in the same region as the scan involving all families (D1S1597). Genotyping additional markers increased the significance of this locus (LOD score=3.5) and refined its position (D1S2672). Several candidate genes of obesity-hypertension are located in close proximity; these include the tumor necrosis factor receptor 2 and atrial natriuretic peptide genes. These results suggest that investigating clinically defined subtypes of hypertension, such as obesity-associated hypertension, may facilitate the search for genes of this complex disorder. (Hypertension. 2005; 46:1280-1285.)

Key Words: hypertension, obesity ■ genetics

Essential hypertension is a heterogeneous disorder that develops because of several overlapping subsets of pathophysiological mechanisms. Obesity is a leading risk factor for the disorder, and as such, may be a key element in one of these subsets. Prospective cohort investigations have demonstrated positive correlations between weight gain and blood pressure (BP) elevation. For example, in the Framingham Study, it was estimated that for each 4.5 kg of weight gain, there is an associated increase in systolic BP of 4 mm Hg in both men and women. Pathophysiological mechanisms linking the increases in adiposity to the elevations in BP are not very well understood. They may include alterations in renal handling of sodium and water, sympathetic nervous system activity, insulin sensitivity, and fatty acid metabolism, with some of these alterations being induced by hormones, growth factors, and cytokines expressed by adipose tissue in response to its expansion.

Hypertension and obesity are multifactorial traits determined by a complex interplay of genes and environments. A number of genes contribute to the determination of each of these traits, and their actions are simple, additive, or more complex, characterized by phenomena such as epistasis and pleiotropy. Furthermore, hypertension and obesity are characterized by etiologic heterogeneity (ie, distinct sets of genes may contribute to their development in different individuals). Moreover, consistent with the above-described link between obesity and hypertension, some genes regulating adiposity or being induced by augmented adiposity may also contribute to the development of hypertension in overweight and obese individuals. As such, these genes may be specific to obesity-associated hypertension.

The complex nature of obesity and hypertension has made it rather difficult to identify genes responsible. Numerous genome-wide scans have provided mostly inconsistent results, with mainly suggestive and only a few significant loci. These outcomes have been largely attributed to inadequate sample sizes and genetic heterogeneity attributable to either
Based on these results, we concluded a subset of hypertensive families with a high prevalence of higher in a set of families selected for hypertension than in a degree of relatedness, and thus genetic homogeneity, is the Saguenay/Lac-St-Jean (SLSJ) region of Quebec (Canada), the genetic investigation into complex traits.

Genetic heterogeneity may substantially improve the power of suggests that, indeed, reducing ethnic and disease-etiology differences. The importance of genetic heterogeneity has been demonstrated recently when genome-wide scan for loci of hypertension, including >6000 individuals of multiple ethnic backgrounds, did not yield any loci with a logarithm of odds (LOD) score >2. On the other hand, genome-wide scan for loci of stroke performed in a smaller sample (n<500) but recruited from a genetically homogeneous Icelandic population identified a highly significant locus with an LOD score of 4.4. Subsequent fine mapping of this locus found a strong association between the gene encoding phosphodiesterase 4D and specific clinical subtypes of ischemic stroke. This carefully conducted study suggests that, indeed, reducing ethnic and disease-etiology genetic heterogeneity may substantially improve the power of genetic investigation into complex traits.

Consistent with the above studies, we demonstrated previously that in a geographically remote population of the Saguenay/Lac-St-Jean (SLSJ) region of Quebec (Canada), the degree of relatedness, and thus genetic homogeneity, is higher in a set of families selected for hypertension than in a set of families selected at random, and is further increased in a subset of hypertensive families with a high prevalence of obesity (Figure 1). Based on these results, we concluded that investigating clinically defined subsets of hypertension may facilitate the search for their specific genes, and that obesity-associated hypertension may, in part, be genetically distinct from hypertension in nonobese individuals. The aim of the present study is to examine these hypotheses further and perform genome-wide scans in the complete set of hypertensive families and in the subset of these families with a high prevalence of obesity.

**Methods**

**Subjects**
The description of families studied here and the population from which they were drawn has been provided previously. In brief, the families were selected from a geographically remote population of French-Canadian origin living in the SLSJ region. The population originates from ancestors of French descent who migrated to this region in the early 19th century. The population has experienced high intrinsic growth, from 5200 inhabitants in 1852 to 285 000 at present. Because of the founder effect, the prevalence of several recessive disorders is higher in the SLSJ than in other populations, and limited allelic diversity exists among patients with these disorders.

The families were selected on the basis of having ≥2 siblings with hypertension (onset at ≤55 years of age) and dyslipidemia. Hypertension was defined as having diastolic BP ≥90 mm Hg on 2 occasions or currently taking antihypertensive medication, with hypertension being documented in medical records. Dyslipidemia was defined as having serum levels of total cholesterol ≥5.2 mmol/L or HDL cholesterol ≤0.9 mmol/L. Dyslipidemia was chosen as a selection criterion because of its potential role in the pathogenesis of essential hypertension; family-based studies in Utah suggest that dyslipidemia and hypertension with an onset at ≤60 years of age are manifestations of a distinct familial syndrome (ie, “familial dyslipidemic hypertension”). Additional selection criteria were the absence of: (1) secondary hypertension, (2) diastolic BP ≥110 mm Hg on BP-lowering medication, (3) gross obesity (body mass index [BMI] >35 kg/m²), (4) diabetes mellitus (fasting blood glucose >6 mmol/L or use of insulin or oral hypoglycemic agents), (5) renal dysfunction (serum creatinine >180 mmol/L), (6) liver disease, (7) malignancy, (8) pregnancy, and (9) substance abuse, including alcohol. To ensure genetic homogeneity, we investigated only families with both parents of Catholic, French-Canadian origin born in the SLSJ region. Once ≥2 siblings in a family satisfied these selection criteria, other siblings, parents, uncles, aunts, and children, not necessarily hypertensive, were also enrolled in the study. The present work is part of an ongoing large collaborative project aimed at identifying genes of essential hypertension in 2 ethnically different populations, namely, French Canadians and African Americans.

With the above criteria, we collected 55 French-Canadian extended families with a total of 389 individuals and an average propositus-sibship size of 5 siblings. From these families, a clinically defined subset of 15 families was selected on the basis of having a high (>70%) prevalence of obesity within propositus sibs. As in our previous studies, obesity was defined by having BMI ≥27 kg/m². This definition was chosen on the basis of a former epidemiological investigation demonstrating that Canadian adults with BMI ≥27 kg/m² have nearly twice the prevalence of hypertension than those with BMI <27 kg/m². In the selected “obese” families, the average sibship size was 4.6 individuals. In the remaining 40 “nonobese” families, average sibship size was 5.2 individuals (Table 1). The degree of genetic homogeneity was evaluated by coefficients of inbreeding and kinship computed with ascending genealogies as described previously.

All 389 individuals were genotyped with 363 microsatellite DNA markers evenly distributed throughout the 22 human autosomes ( Cooperative Human Linkage Center Screening Set; version 6.0); the density of DNA markers was <10 cM. Subject DNA was extracted using a standard protocol and genotyped at the Whitehead Institute for Biomedical Research/Massachusetts Institute of Technology Center for Genome Research and McGill University and Genome Quebec Innovation Centre. For the Boston and Montreal genotyping, we used PEDMANAGER version 0.9 (Mark J. Daly, personal communication, 2004) for allele binning, inheritance checking, and estimating allele frequencies. Genome-wide scans were performed with qualitative multipoint linkage analysis (GeneHunter 2.1) in the entire set of hypertensive families and in the “obese” and “nonobese”...
subsets of these families.21 The software allows the extraction of complete inheritance information from pedigrees of moderate size; this information is then used in nonparametric linkage analysis.21 In the present study, the nonparametric linkage “all” scoring function was used. Most pedigrees were small enough to be used by GeneHunter without cutting. In all linkage analyses, a maximum pedigree size was set to max bits = 16 (the default setting). “Max bits” is the number of bits in the inheritance vector (the number of meioses being examined) and is calculated as 2N − F, where N is the number of nonfounders and F is the number of founders in a pedigree. When a pedigree exceeded the size limit, the software selected the least informative individuals and excluded them from the analysis.21 Affected status was defined as: (1) being hypertensive (hypertension), (2) having BMI ≥ 27 kg/m² (obesity), or (3) being hypertensive and having BMI ≥ 27 kg/m² (obesity-associated hypertension).20 Stringent although widely accepted thresholds of LOD score > 1.9 and LOD score > 3.3 have been used to claim “suggestive linkage” and “significant linkage,” respectively, with a genome-wide \( P < 0.05 \).22,23

The study was approved by an institutional review committee, and all subjects gave informed consent.

Results

Clinical Characteristics of Hypertensive Families

Families included in this study are those investigated in our previous genealogical study;13 their genealogies were reconstructed from propositus sibships. The whole set of 55 hypertensive families included 389 individuals, of whom 211 were hypertensive (Table 1). On average, the hypertensive individuals were 54.8 ± 0.6 years of age and moderately overweight; their mean fasting insulin and triglyceride serum levels were within the normal range, and their average total cholesterol was borderline high (Table 2). From within the set of all 55 hypertensive families, a subset with “high” prevalence of obesity (n=15) was identified. This subset included 57 hypertensive individuals at the propositus-sibship level; when compared with 154 hypertensive subjects from the remaining nonobese families (n=40), these individuals were more obese (as expected), but they did not differ by gender, age, and fasting serum triglycerides, cholesterol, and insulin concentrations. They also did not differ in the number of BP-lowering medications prescribed per individual, suggesting that the severity of the disease was similar in the 2 subsets of hypertensive subjects (Table 2).

Analysis of Ascending Genealogies

We demonstrated previously that the degree of genetic homogeneity, as assessed by analyses of ascending genealogies, is increased in a subset of “obese” hypertensive families (n=15) compared with the entire set of hypertensive families (n=55).13 In the present study, we extended this finding in that we have shown that the degree of genetic homogeneity in this subset is higher than that in the remaining “nonobese” families (n=40; Figure 1).

Genome-Wide Scan

In the whole set and the “obese” subset of hypertensive families, the loci with the highest LOD score for hypertension were identified in the same 2 chromosomal regions, namely 1p36 and 11p15 (Figure 2). In the former region, the LOD score was 2.3 in all families and 2.5 in “obese” families. This region also contained the most significant locus for obesity in all and “obese” families, with the LOD score being 1.6 in the former group but 2.9 in the latter group of families. Furthermore, when affected status was set to be hypertensive and obese (obesity-associated hypertension) rather than either hypertensive or obese, the LOD score increased in both groups of families; it was 1.9 in all families and 3.1 in “obese” families. No significant or suggestive loci of hypertension or obesity or obesity-associated hypertension were identified within the 1p36 chromosomal region in the remaining “nonobese” families. The LOD score of 3.1 was the most significant of differences between obese and nonobese families.

### TABLE 1. No. of Affected and Nonaffected Individuals Within all Hypertensive Families and Within “Obese” and “Nonobese” Subsets of These Families

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>All Families (n=55)</th>
<th>“Obese” Families (n=15)</th>
<th>“Nonobese” Families (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Affected</td>
<td>Nonaffected</td>
<td>Affected</td>
</tr>
<tr>
<td>Propositus sibships</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>211</td>
<td>66</td>
<td>57</td>
</tr>
<tr>
<td>Obesity</td>
<td>153</td>
<td>124</td>
<td>62</td>
</tr>
<tr>
<td>Hypertension with obesity</td>
<td>123</td>
<td>154</td>
<td>52</td>
</tr>
<tr>
<td>Nonpropositus sibships</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>77</td>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td>Obesity</td>
<td>44</td>
<td>68</td>
<td>6</td>
</tr>
<tr>
<td>Hypertension with obesity</td>
<td>28</td>
<td>84</td>
<td>4</td>
</tr>
</tbody>
</table>

### TABLE 2. Clinical Characteristics of Genotyped Hypertensive Subjects at the Propositus-Sibship Level of Selected Pedigrees

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>All Families</th>
<th>Obese Families</th>
<th>Nonobese Families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>94/117</td>
<td>26/31</td>
<td>68/86</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.8±0.6</td>
<td>53.7±0.8</td>
<td>55.2±0.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2±0.3</td>
<td>30.7±0.5*</td>
<td>27.2±0.4</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.6±0.1</td>
<td>5.5±0.1</td>
<td>5.6±0.1</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.3±0.1</td>
<td>2.4±0.2</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>121.2±4.4</td>
<td>130.0±9.0</td>
<td>118.7±5.0</td>
</tr>
<tr>
<td>No. of BP-lowering drugs</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
</tr>
</tbody>
</table>

*Statistical significance of differences between obese and nonobese families \( P < 0.0001 \).
significant obtained in this study; however, the locus was located at the end of the genotyped region of chromosome 1 and, as such, could represent a spurious result (Figure 2). Therefore, we genotyped 7 additional microsatellite markers in all members of the “obese” families. Three of these markers were added to extend the mapped region telomERICally, and 4 of them were included to increase the density of markers in the region of the identified locus of obesity-associated hypertension. This additional genotyping augmented the significance of the locus to LOD = 3.5 and refined its position (D1S2672; Figure 3). Together, the above results suggest that the locus on chromosome 1p36 contains a gene (or genes) that increases susceptibility to the development of hypertension in obese individuals; as such, the gene(s) may be specific to obesity-associated hypertension.

The pattern of results on chromosome 11p15 was different from that on chromosome 1p36. In all and “obese” families, an LOD score of 2.5 was obtained when affected status was defined as being hypertensive (hypertension). When it was defined as being obese (obesity) or hypertensive and obese

Figure 2. Genome-wide linkage analyses of hypertension (blue), obesity (yellow), and obesity-associated hypertension (red) in “all” hypertensive families and in “obese” and “nonobese” subsets of these families. Plots of the multipoint LOD scores from chromosomes with an LOD score ≥ 1.9 are shown. H indicates hypertension; O, obesity; OH, obesity-hypertension.
Gene linkages between hypertension (blue), obesity (yellow), and obesity-associated hypertension (red) and the 1p36 locus. Linkage analyses were performed with additional genotyping in “obese” hypertensive families.

(Obesity-associated hypertension), no significant loci in the region were observed in either all or “obese” families. A similar trend was also observed in “nonobese” families, although the LOD score for hypertension did not reach the level of “suggestive” linkage (Figure 2). These results indicate that chromosome 11p15 may include a gene(s) that increases an individual’s vulnerability to hypertension independently of obesity.

Discussion
Genetic approaches to complex disorders, such as hypertension and obesity, provide an excellent opportunity to improve our understanding of their underlying mechanisms.9 Although these approaches have been highly successful in uncovering the mechanisms of monogenic disorders, their use in case of complex polygenic diseases has proven to be more difficult than originally expected. Most of the difficulties have been attributed to genetic (ie, ethnic and disease-etiology) heterogeneity of cohorts studied.9 Accordingly, the results of the present investigation suggest that, indeed, performing genome-wide scans in pedigrees with familial occurrence of clinically defined subtypes of hypertension, such as obesity-associated hypertension, may facilitate the search for genes of the disease. As a corollary, the results also suggest that obesity-associated hypertension may be, in part, genetically distinct from hypertension in nonobese individuals. More specifically, we showed that genome-wide scan, performed in either the entire set of hypertensive families or the subset of families with a high prevalence of obesity, identified most significant loci of hypertension in the same 2 chromosomal regions: 1p36 and 11p15. Despite the difference in sample size, the observed LOD scores were either modestly higher in the subset of “obese” hypertensive families than in the entire set of hypertensive families (1p36), or they did not differ between the 2 groups (11p15). Moreover, when affected status was changed from “hypertension” to “obesity-associated hypertension,” the significance of the 1p36 locus increased in the subset of obese families and decreased in the entire set of families; for the 11p15 locus, the change decreased the significance in both groups of families (Figure 2). Additional genotyping of the 1p36 locus further augmented its significance for obesity-associated hypertension to an LOD score of 3.5. These results indicate that the 1p36 locus may contain a gene that is specific to obesity-associated hypertension, and the 11p15 locus may include a gene of hypertension that, in contrast, acts independently of obesity.

Lander and Kruglyak proposed that linkage results must be replicated to be credible.22 Importantly for the findings of the present study, several previous investigations have demonstrated suggestive and significant linkages (LOD scores >1.9 and >3.3, respectively22) of obesity and hypertension to the 1p36 locus. All these studies, similar to the current one, have been performed in white populations of European origin, indicating that the locus may be specific to this ethnic group. In greater detail, a quantitative trait locus of BMI was mapped to 1p36 in 2 large genome-wide scans with LOD scores of 2.223 and 2.5.24 Furthermore, a study examining a 20-cM region of 1p36 detected significant linkage and association between a microsatellite polymorphism in the tumor necrosis factor receptor 2 gene and hypertension.25 Interestingly, the selection criteria of that investigation were quite similar to those of the current study (ie, the subjects were ascertained on the basis of familial occurrence of early-onset hypertension and no diabetes mellitus). Subsequently, a genome-wide scan was performed in the same cohort that identified 1p36 as 1 of 2 most significant loci of hypertension.26 A 1-LOD drop region around the peak of the 1p36 locus contains several candidate genes potentially involved in the pathogenesis of obesity-associated hypertension; these include the tumor necrosis factor receptor 2 gene and the atrial natriuretic peptide gene. Both genes encode peptides that can be produced by adipose tissue, and that can influence adipogenesis and lipogenesis in an autocrine/paracrine manner; in addition, when released into the circulation, they may regulate BP.25–27–29 As such, either of the 2 genes may exert pleiotropic effects on adiposity and BP. Alternatively, the locus may include 2 closely linked genes that cosegregate within families, with one being involved in the regulation of adiposity and the other in the control of BP.

In the present study, the 11p15 locus demonstrated suggestive linkage to hypertension that was not associated with obesity. Consistently, a suggestive quantitative trait locus of exercise diastolic BP adjusted for age, gender, and BMI was detected previously in the same chromosomal region.30 However, further investigations are necessary to obtain significant linkage and, thus, confirm the existence of this locus.

Perspectives
Our previous genealogical research in a geographically isolated population of French-Canadian origin has demonstrated that the degree of genetic homogeneity increases by ascertaining families with a high prevalence of hypertension, and that it further augments by selecting a subset of these families with a high prevalence of obesity-associated hypertension.13 These results suggest that conducting genome-wide scans of clinically defined subtypes of hypertension, such as obesity-associated hypertension, is likely to facilitate the search for
genes of the disease, and that hypertension in obese and overweight individuals may be, in part, genetically distinct from that in nonobese individuals. The results of the present study performed in the same sets of hypertensive families support these conclusions: (1) Despite the reduced sample size, “significant” linkage was detected for obesity-associated hypertension, and only “suggestive” linkage was observed for hypertension. (2) Some loci appear to be specific to obesity-associated hypertension, whereas others do not. Together, we believe that our research indicates that reducing genetic heterogeneity of essential hypertension by studying its subcategories and by investigating ethnically homogeneous cohorts may improve the power to identify genetic causes of the disease.

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References

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