A genome wide scan for early onset primary hypertension in Scandinavians

Fredrik von Wowern¹, Kristina Bengtsson¹,², Cecilia M. Lindgren¹, Marju Orho-Melander¹, Frej Fyhrquist³, Ulf Lindblad², Lennart Råstam², Carol Forsblom³, Timo Kanninen¹, Peter Almgren¹, Philippe Burri¹, Per Katzman⁴, Leif Groop¹, U. Lennart Hulthén¹ and Olle Melander¹,*

¹Department of Endocrinology and ²Department of Community Medicine, Malmo¨ University Hospital, SE-205 02, Malmö, Sweden, ³Department of Medicine, Division of Internal Medicine, Helsinki University Central Hospital, FI-00029, Helsinki, Finland and ⁴Department of Medicine, Hospital of Helsingborg, SE-251 87 Helsingborg, Sweden

Received May 12, 2003; Revised and Accepted June 15, 2003

With the aim of identifying hypertension susceptibility loci, we performed a genome wide scan in Scandinavian sib-pairs with early onset primary hypertension. To be classified as affected, a diagnosis of primary hypertension at age ≤ 50 years was required. Two hundred and forty three patients with onset of primary hypertension at 40.0 ± 7.7 (mean ± SD) years from 91 families (91 sib-ships with a mean of 2.7 and a range of 2–6 affected members per sib-ship) were genotyped with 362 microsatellite markers with a density of ~10 cM. Loci obtaining nominal \( P \leq 0.016 \) (LOD score \( \geq 1.0 \)) were fine mapped with additional markers. Multipoint non-parametric linkage analysis was performed using GENEHUNTER v 2.0. Using simulations, a nominal \( P \leq 0.0002 \) was determined to be a genome wide significant evidence of linkage. In the 10 cM genome wide scan, nominal \( P \leq 0.016 \) were found on chromosomes 1 at 81 cM (\( P = 0.007 \)), 2 at 115 cM (\( P = 0.006 \)), 3 at 108 cM (\( P = 0.006 \)), 14 at 45 cM (\( P = 0.0002 \)) and at 99 cM (\( P = 0.001 \)), 17 at 42 cM (\( P = 0.015 \)) and 19 at 89 cM (\( P = 0.007 \)). After fine mapping of these loci, one of the chromosome 14 loci just obtained the level of genome wide significance (\( P = 0.0002 \) at 41 cM) and the chromosome 2 locus reached suggestive evidence of linkage (\( P = 0.002 \) at 118 cM). Our data suggest a hypertension susceptibility locus on chromosome 14 around 41 cM. The locus on chromosome 2 is also promising as it has been implicated in hypertension and blood pressure regulation in earlier genome scans.

INTRODUCTION

Primary hypertension is a multifactorial disease with largely unknown etiology and pathophysiology. The disease most likely results from an interaction between a number of genetic and environmental factors. Twin and family studies have suggested that 30–60% of the blood pressure variability in the general population is determined by genetic factors (1,2). In families from Utah, individuals with two or more hypertensive first degree relatives developed hypertension approximately four times as often by the age of 40 years, three times as often by the age of 50 years and twice as often by the age of 60 years, when compared to individuals with no such family history. The predictive value of a family history of hypertension was negligible for hypertension with an age at onset by the age of 70 years (3). This implies that the importance of genetic factors for the development of hypertension is greatest in patients with an early onset of the disease.

The identification of the genetic defects in a number of rare monogenic forms of early onset salt sensitive hypertension now allows for early diagnosis, prevention with salt restriction and specific and effective pharmacological treatment of these forms of hypertension (4). The genetic background of primary hypertension is more complex and considerably harder to dissect as the number of genes and environmental factors involved is greater, resulting in a ‘non-Mendelian’ inheritance pattern. However, lessons learned from the monogenic forms of hypertension have highlighted what the clinical benefits could be if susceptibility genes were to be identified also for primary hypertension.
Studies of candidate genes for hypertension have been limited to genes coding for proteins involved in known blood pressure regulatory systems. In contrast, genome wide scans for hypertension do not presume any prior knowledge of the mechanisms underlying blood pressure regulation. Genome wide scanning followed by positional cloning has recently proven fruitful in identifying disease susceptibility genes for wide scanning followed by positional cloning has recently been shown to be a powerful approach for identifying disease susceptibility genes in familial aggregation studies (5). Still, no primary hypertension susceptibility gene has been identified by this method but a number of promising chromosomal regions showing evidence of linkage to hypertension or blood pressure variation have been identified (2, 7-21).

In an attempt to identify hypertension susceptibility loci, we performed a genome wide scan with 10 cM microsatellite marker intervals in Scandinavian sib-pairs with early onset primary hypertension. Loci obtaining a log_{10} of the likelihood ratio for linkage (LOD score) of $\geq 1.0$ ($P \leq 0.016$) were then fine mapped with additional markers to a mean density of 4.6 cM (range 3-5 cM).

RESULTS

Simulations for genome wide significance thresholds

One thousand genome wide scans were simulated in order to obtain appropriate thresholds for genome wide significant and suggestive evidence of linkage. The genome wide threshold for suggestive linkage was determined to be a $P$-value of $\leq 0.003$ (LOD score $\geq 1.7$) and for genome wide significant linkage, at the 5% level, a $P$-value of $\leq 0.0002$ (LOD score $\geq 2.7$).

Genome wide scan and fine mapping

In the 10 cM basic genome wide scan, nominal $P$-values $\leq 0.016$ (LOD score $\geq 1$) were found on chromosomes 1 at 81 cM ($P = 0.007$), 2 at 115 cM ($P = 0.006$), 3 at 108 cM ($P = 0.006$), 14 at 45 cM ($P = 0.0002$) and at 99 cM ($P = 0.001$), 17 at 42 cM ($P = 0.015$) and 19 at 89 cM ($P = 0.007$).

All genetic regions between the flanking markers of the loci with LOD scores $\geq 1.0$ were subjected to fine mapping with additional microsatellite markers (www.endo.mas.lu.se/html/pub/gws_allmarkers.txt) to reach an average marker density of 4.6 cM (range 3-5 cM) with an average marker heterozygosity index of 0.78. The flanking markers of each region of linkage covered at least the 1-LOD support interval, which has been suggested to contain the gene responsible for the linkage with $\sim 90\%$ probability (22). After fine mapping, one region on chromosome 14, around 41 cM, just obtained genome wide significance and the region of linkage from the basic scan was narrowed down (Table 1 and Fig. 1). In addition, the strength of the linkage on chromosome 2 increased and reached suggestive evidence of linkage (Table 1 and Fig. 1). The LOD scores of 2.7 on chromosome 14 and 1.8 on chromosome 2 correspond to non-parametric linkage scores of 3.5 and 2.9, respectively. No other regions exceeded suggestive evidence of linkage. Using marker allele frequencies of a population of 1200 Finns (data obtained from the Finnish Genome Center) in the linkage analysis, instead of founder allele frequencies, did not change LOD scores by more than, at most, 3%.

DISCUSSION

We undertook a genome wide scan, followed by fine mapping of regions with LOD scores $\geq 1.0$, with the aim of identifying susceptibility loci for early onset primary hypertension. Several phenotypic considerations were made when designing this study. The importance of genetic factors in primary hypertension is greater the lower the age at onset of the disease (3). In an attempt to enrich the genetic component of the disease and to reduce the number of phenocopies, we therefore only included patients with an age at onset of primary hypertension $\leq 50$ years. Another important prerequisite for successful mapping of disease susceptibility genes is a reliable phenotype. Genome wide scans for blood pressure variation in the general population, as compared to genome wide scans using the dichotomous trait of ‘hypertension’ as the phenotype, have the advantage that the quantitative information of the blood pressure distribution can be used, which may yield a greater power. On the other hand, with some exceptions (2), genome scans using the phenotype of blood pressure as a quantitative trait usually have to rely on few blood pressure measurements taken at a single examination which may result in a phenotype less reliable than diagnosed hypertension, as office blood pressure measurements suffer from a certain degree of intra-individual variability (23). In order to maximize the reliability of the phenotype in the present study, all the patients classified as being affected were required to have a diagnosis of primary hypertension (based upon at least three diastolic blood pressure recordings $>90$ mmHg on different occasions) and to have begun chronic antihypertensive medication at $\leq 50$ years of age. As the guidelines at the time when the patients were diagnosed (24) required additional diastolic blood pressure recordings of $>95$ mmHg before initiation of antihypertensive medication, the number of mis-classified subjects is likely to be very small.

Table 1. Genome wide scan regions showing significant or suggestive evidence of linkage

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Markers flanking peak</th>
<th>Marker closest to peak stage I/stage II</th>
<th>Peak position$^a$ (cM) stage I/stage II</th>
<th>LOD score stage I/stage II</th>
<th>$P$-value$^b$ stage I/stage II</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>D2S2216-D2S347</td>
<td>D2S160/D2S437</td>
<td>115/118</td>
<td>1.4/1.8</td>
<td>0.006/0.002</td>
</tr>
<tr>
<td>14</td>
<td>D14S70-D14S63</td>
<td>D14S288/D14S288</td>
<td>45/41</td>
<td>2.7/2.7</td>
<td>0.0002/0.0002</td>
</tr>
</tbody>
</table>

$^a$Genetic distance from beginning of chromosome.

$^b$Nominal $P$-value (unadjusted for multiple comparisons).

Stage I = results after the 10 cM genome wide scan.

Stage II = results after fine mapping.
We found genome wide significant evidence of linkage to early onset primary hypertension at 41 cM on chromosome 14 (Table 1 and Fig. 1). No previously published hypertension or blood pressure genome wide scan has detected genome wide significant linkage within the 1-LOD support interval of this peak (37–46 cM). However, nominal evidence of linkage was reported around marker D14S306, at 44 cM, for diastolic blood pressure in a genome scan of Mexican American families (14). Despite the weaker evidence of linkage in Mexican Americans, the co-existence of linkage in two independent studies strengthens the support of this region as a hypertension susceptibility locus. Although the phenotype in the study of Mexican Americans was variation in diastolic blood pressure and our phenotype was early onset primary hypertension, the diagnosis of the phenotype in the study of Mexican Americans was variation in diastolic blood pressure and our diagnostic criteria were acute primary hypertension at least three consecutive blood pressure measurements of >160 mmHg systolic blood pressure and/or >90 mmHg diastolic blood pressure on different occasions) ≤50 years; (ii) initiation of chronic antihypertensive medication at age ≤50 years; and (iii) the proband should have at least one affected sibling. Probands were identified from the health care files and invited to a re-examination during which the inclusion criteria were confirmed. Via the probands, relatives were contacted and examined. In the community of Skara, one of the recruiting centers in southern Sweden, the prevalence of hypertension, as defined by the first two affection status criteria, is 3% in the age span of 40–50 years (U. Lindblad, personal communication).

Patients with secondary hypertension were excluded. Extensive work-up for secondary hypertension was initiated when indicated by any of the following clinical signs: elevated serum creatinine, hypokalemia, albuminuria, hematuria, inability...
to control blood pressure with ≥2 antihypertensive agents and symptoms of pheochromocytoma.

The proband of the hypertensive sib-ships was always non-diabetic. However, as primary hypertension is characterized by insulin resistance and often by overt type 2 diabetes, we also included hypertensive siblings with type 2 diabetes provided that they were normoalbuminuric and had normal serum creatinine values. In total, 21 out of the 243 affected patients (8.6%) were type 2 diabetic. None of the patients had type 1 diabetes.

Signs of alcoholism were evaluated by a careful anamnesis and measurement of serum amino-transferases. Subjects drinking more than 70 cl of strong liquor/week or having impaired liver function (as indicated by elevated amino-transferases) were excluded. Altogether, 243 affected patients from 91 families (91 sib-ships with a mean of 2.7 and a range of 2–6 affected members per sib-ship) were ascertained. The age at onset of hypertension was 40.0 ± 7.7 years (mean ± SD), age at the time of the study was 57.9 ± 10.1 years and body mass index (BMI) was 27.4 ± 4.4 kg/m². The blood pressures measured at the study examination were 153 ± 21 mmHg (systolic) and 90 ± 11 mmHg (diastolic) and represent ‘on treatment’ values as all affected patients were on chronic antihypertensive treatment.

In total, 54 parents were genotyped. For eight sib-ships, both parents were available for genotyping; for 38 sib-ships, genotype information was available on one of the parents and for the remaining 45 sib-ships, none of the parents were genotyped. As the affected sib-pair analysis was applied, the affection status of the parents was not taken into account but their genotypes were used to determine identity by descent in the affected offspring. An additional 129 unaffected siblings to affected patients were genotyped in order to increase phase information in affected sib-ships from families where one or both parents were unavailable for genotyping.

Blood pressure was measured with a mercury sphygmomanometer in the supine position after 10 min rest by specially trained nurses and physicians. BMI was calculated as the ratio of the weight in kilograms to the square of the height in meters.

Genotyping

Total genomic DNA was extracted from venous blood by standard methods. Genotyping was performed at the Finnish Genome Center, Helsinki, Finland. A set of 362 microsatellite markers (average spacing ~10 cM) from Applied Biosystems (Foster City, CA, USA) was used (‘Applied Biosystems Linkage Mapping Set MD10’). Additional markers for fine mapping of regions with a LOD score ≥1.0 as well as all marker map positions (sex averaged genetic distance from the beginning of the chromosome) were obtained at http://research.marshfieldclinic.org/genetics/About_Marshfield/marshfield.htm. All genetic distances reported in this paper are according to this map. All markers genotyped can be found at (www.endo.mas.lu.se/html/pub/gws_allmarkers.txt (see Supplementary Material). Genotyping was performed using polymerase chain reaction (PCR) with fluorescently labeled primers according to conditions supplied by the manufacturer (Applied Biosystems), followed by capillary electrophoresis on a MegaBase 1000 instrument (Molecular Dynamics, Sunnyvale, CA, USA). The capillary read-length is 40 cm. Sample injection is electrokinetic and after 5 min prerun (10 kV) samples are run for 65 min at 44°C. Allele-calling was done using ‘Genetic Profiler’ software (Molecular Dynamics) and data was checked for Mendelian errors with PEDMANAGER (Whitehead Institute for Biomedical Research, MIT, Cambridge, MA) and PEDCHECK software (25).

Statistical analysis

As the inheritance pattern of primary hypertension is unknown, evidence of linkage was assessed with a non-parametric method using GENEHUNTER software version 2.0 (26). This software includes all the functions of MAPMAKER/SIBS (27). Complete multipoint analysis of the statistical significance of allele sharing identical by descent among all affected sib-pairs at each location in the genome was performed. The contribution of each sib-pair was weighted to compensate for the difference in the size of the sib-ships, using GENEHUNTER v 2.0 (26). The strength of the linkage was expressed as LOD score and P-value. If allele sharing identical by descent in sib-pairs could not be directly determined (e.g. when DNA from one or both parents was missing), parental haplotypes were estimated based on the haplotypes of their offspring using GENEHUNTER v 2.0 (26). Marker allele frequencies used in the analysis were those of the founders of the families in the study (e.g. the parents of the hypertensive sib-ships) (26). If the founders were not available for genotyping, their allele frequencies were estimated based on the allele frequencies of their offspring using PEDMANAGER (Whitehead Institute for Biomedical Research, MIT, Cambridge, MA). A confirmatory analysis was performed using marker allele frequencies derived from a population of 1200 Finns (data obtained from the Finnish Genome Center). Full-sib status of all siblings was confirmed using the computer program RELATIVE (28).

According to suggested guidelines, to interpret the results of linkage analysis in a genome wide scan (29), a nominal P-value of ≥2 × 10⁻³ (LOD score ≥3.6) should be considered as statistically significant and a nominal P-value ≤7 × 10⁻⁴ (LOD score ≥2.2) as suggestive evidence of linkage at the genome wide level. However, these thresholds assume complete informativeness and an infinite marker map density. To establish appropriate thresholds for genome wide suggestive and significant linkage for our particular set of data, considering the more sparse marker map density and lack of total informativeness, 1000 simulations were performed by generation of artificial genotypes in our particular set of families [GENSIM software (M. J. Daly, unpublished data)]. These simulations matched our data set with respect to marker density, marker informativeness, individuals genotyped, affection status and fraction of missing data.

World wide web links

The genetic distances between markers obtaining the best LOD scores in our study and other studies was obtained by marker maps available at http://research.marshfieldclinic.org/genetics/About_Marshfield/marshfield.htm. Linked regions were searched for positional candidate genes at http://www.ncbi.nlm.nih.gov/mapview/maps.cgi.
SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online and also at www.endo.mas.lu.se/html/pub/gws_allmarkers.txt.

ACKNOWLEDGEMENTS

We thank Dr Mats Ekberg and Dr Urban Lantz for their valuable help in the collection of families and Lena Rosberg, Anna Berglund, Ingrid Wetterholz, Ann Carlsson and Lena Lundin for excellent technical assistance. Genotyping was performed at the Finnish Genome Center, University of Helsinki, Helsinki, Finland. The Finnish Supercomputing Center is acknowledged for the computational capacity used in the simulations. The study was supported by grants from the Swedish Medical Research Council, the Swedish Heart and Lung Foundation, the Medical Faculty of Lund University, Malmö University Hospital, Skaraborg Institute, the Skaraborg County Council, the Albert Pålsson Research Foundation, the Crafoord Foundation, the Ernhold Lung Foundation, the Medical Faculty of Lund University, the Swedish Medical Research Council, the Swedish Heart and Lung Foundation, the University of Helsinki, Helsinki, Finland. The Finnish Supercomputing Center is acknowledged for the computational capacity used in the simulations. The study was supported by grants from the Swedish Medical Research Council, the Swedish Heart and Lung Foundation, the Medical Faculty of Lund University, Malmö University Hospital, Skaraborg Institute, the Skaraborg County Council, the Albert Pålsson Research Foundation, the Crafoord Foundation, the Ernhold Lung Foundation, the Medical Faculty of Lund University, Malmö University Hospital, Skaraborg Institute, the Skaraborg County Council, the Albert Pålsson Research Foundation, the Finnish Academy and the Region Skane.

REFERENCES