Genome-Wide Scan for Hypertension in Sydney Sibships: The GENIHUSS Study

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We report here the results of the GENIHUSS study (GENetic Investigation of Hypertension Undertaken in Sydney Sibships)—a genome-wide scan to identify loci linked to essential hypertension (HT). Subjects were Anglo-Celtic Australian sibpairs resident in or near Sydney, Australia, with onset of HT before age 60 years (mean, 44 ± 13 SD years). A 10-cM scan involving 400 microsatellite markers and 252 HT sibpairs was followed by fine mapping of the most promising locus using 296 HT sibpairs (481 individuals from 200 families). Multipoint and two-point nonparametric linkage analyses were performed using MAPMAKER/SIBS, GENEHUNTER II, and SPLINK. Suggestive loci were found on chromosomes 1 (4 cM) and 4 (129 cM). The chromosome 4 locus coincided with a QTL for systolic blood pressure (BP) in the Australian Victorian Family Heart Study, and the locus on chromosome 1 contains the chloride channel gene CLCNKB and tumor necrosis factor receptor 2 gene TNFRSF1B, which have each shown association with HT. Our study adds to findings of HT loci emanating from genome scans. Am J Hypertens 2005;18:828–832 © 2005 American Journal of Hypertension, Ltd.

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Essential hypertension (HT) is the sum result of the individual small effect of polymorphisms in an as yet unknown number of genes that influence blood pressure (BP) in response to particular environmental influences. To systematically identify all of the major genetic loci responsible the approach of genome scanning has been thought to offer considerable promise. Therefore we conducted a genome-wide scan for HT in affected sibships from Sydney.

Methods

Subjects

All subjects were Anglo-Celtic white Australians of British ancestry who were resident in Sydney and adjacent regions. Families were recruited from 1992 to 2003 by advertising in community newspapers, and through posters and leaflets sent to general practitioners (GPs), pharmacists, and medical centers in Sydney. Subjects had to have HT themselves, defined as systolic/diastolic BP of >140/90 mm Hg, as well as having two or more full siblings who were also HT. Diagnosis of HT had to be before age 60 years and the subjects had to be confirmed as being free of secondary HT, diabetes, heart and kidney disease by their GP, who confirmed details in a questionnaire completed by each subject. A 50-mL blood sample was collected, and height and weight, measured by conventional devices, were used to calculate body mass index (BMI). The study was approved by the University of Sydney Ethics Committee and informed consent was obtained from each participant at recruitment.

There were a total of 296 affected sibpairs (ASPs) from 200 families that included 140 duos, 44 trios, 12 quartets, 3 quintets, and 1 sextet (= 481 individuals), and which were weighted as described. The genome-wide scan involved 252 of these ASPs (118 duos, 35 trios, 11 quartets, 3 quintets, 1 sextet; 406 individuals), with the full 296 being used for fine-mapping. Given 75% parental heterozygosity this number had >90% power to detect linkage at logarithm of the odds for linkage (LOD) ≥2 for genetic loci with sibling recurrent risk (λ) values of ~1.6.

Characteristics of the 481 sibs (36% men, 64% women) were (mean ± SD): age, 62.9 ± 10.4 years; age of onset of HT, 43.8 ± 12.7 years; BMI, 27.7 ± 4.9 kg/m²; systolic BP, 171.6 ± 22.8 mm Hg; diastolic BP, 103 ± 11.9 mm Hg.
Genotyping

Genomic DNA was extracted from whole blood using QIAamp DNA Blood Midi Kits (Qiagen, Hilden, Germany). Genotyping used the ABI PRISM Human Linkage Mapping Set, version 2 (Applied Biosystems, Foster City, CA) primers for 400 microsatellite markers distributed at an average density of 10 cM throughout the genome (excluding the Y chromosome), and was performed by the Australian Genome Research Facility, Melbourne, Australia (http://www.agrf.org.au) using ABI 377 (Applied Biosystems) sequencers and robotics. Fine mapping of a peak of interest on chromosome 4 involved an additional 4 markers—D4S2985, D4S1615, D4S2286 and D4S1576—spanning the region 124 to 135 cM (average marker density 2.75 cM). Marker positions were taken from the Ensembl Human Genome Database (http://www.ensembl.org/Homo_sapiens/) and used deCode Genetics (http://www.decode.com/) mapping data.

Statistical Analyses

Evidence of linkage was assessed by nonparametric affected sib pair (ASP) methods. Because the relative power and behavior of different standard nonparametric tests used for complex traits are known to vary,4 we applied several of the better methods4 to test for discordant allele sharing by descent (IBD) or state (IBS). We used GENEHUNTER II, which imputes multipoint IBD linkage and generates a nonparametric, approximately normally distributed, Z score. Another multipoint test used was MAPMAKER/SIBS (MMS), which restricts the maximization of the likelihood ratio of Risch9 to within the possible triangle,6 (discussed later) and generates maximum LOD score (MLS) values with five intervals per genotyped marker, as well as information content and exclusion maps. For this test allele frequencies were estimated using the affected families weighted by number of founders in each pedigree. We also used SPLINK (UNIX version 1.09), which generates IBD estimates using all sibs in a sibship under the constraints imposed by linkage (possible triangle restriction: z[1] < 0.05 and z[0] < 0.5 × z[1], where z[1] and z[0] are sharing of 1 and 0 alleles IBD, respectively) and compares the IBD distribution to that under the constraints imposed by linkage (possible triangle restriction: z[1] < 0.05 and z[0] < 0.5 × z[1], where z[1] and z[0] are sharing of 1 and 0 alleles IBD, respectively) and compares the IBD distribution to that expected under no linkage.7 Both weighted and unweighted SPLINK were applied, where number of weighted sibpairs in a family = number of unweighted sibpairs/2 × number of affected sibs. Two-point linkage was also ascertained by IBS χ² using SIB-PAIR. This test compares observed alleles shared IBS at a marker with what would be observed randomly using contingency table analysis.8 Of the significance thresholds set for acceptance of linkage,3,9–12 the most stringent allows acceptance of only one false positive every 20 genome scans, requiring LOD ≥ 3.6 (P ≤ .000022) for significant linkage and LOD ≥ 2.2 (P ≤ .00074) for suggestive linkage.7 On the other hand, the lower stringencies allowed by others help provide a balance that also controls for false negatives.13 True genetic susceptibility loci are, moreover, likely to be accompanied by positive linkage data for adjacent markers.

Results

Genome-scan linkage results are shown in Fig. 1. No loci attained genome-wide significance at the Lander and Kruglyak3 level. The two most significant peaks (Table 1) were centered at D4S2286 (4q28.3; 129 cM) and D1S408 (1p36.32; 4 cM). Although the scores obtained for these were at best suggestive of linkage, apparent support was nevertheless present for data from flanking markers. Other loci with MMS LOD > 1 were at 8q12.1 and Xp26.1 (LOD = 1.2 and 1.1, respectively).

Fine mapping of the chromosome 4 locus refined the position of the peak to 129 cM (D4S2286) and increased significance (Fig. 2). Fine mapping of the chromosome 1 locus has been performed previously in a separate, non-automated, in-house scan of just this chromosome in overlapping ASPs.14

Discussion

Our genome-wide scan has identified possible HT loci on chromosomes 1 and 4. Unlike most other genome scans for HT we used a variety of statistical tests to assure reliability of results. For example, although multipoint IBD is generally believed to be more powerful, it is sensitive to any genotyping inaccuracy and marker-space specifications, therefore it should not be used alone.

The locus we find centered at the 129-cM position on chromosome 4 corresponds to a QTL for SBP (z = 3.2) at 95 to 132 cM in the Victorian Family Heart Study.15 Importantly, like our study, this involved Australian subjects, meaning similar genes and environment. The chromosome 4 region of interest is, moreover, syntenic with a section of rat chromosome 2 that contains a QTL for systolic BP16,17 and left ventricular mass18 in the spontaneously hypertensive rat. In polygenic conditions linkage peaks may, on average, be indicative of causative genes within approximately 10 cM either side of the peak.19 For this interval around the chromosome 4 peak (120.7 to 142.8 cM) there were approximately 29 known genes and 36 genes of unknown function. One, PDE5A (http://www.ensembl.org/Homo_sapiens/), codes for a cGMP-specific phosphodiesterase that catabolizes cGMP in the vasculature,20 and when inhibited leads to vasodilation,21 reduced platelet aggregation,22 and a decreased arterial pressure.23–25

The peak at 1p36.32 has shown suggestive linkage (LOD = 2.5) to systolic BP in HT Hispanic families,26 as well as HT in Taiwanese27 and Sardinians.28 This HT locus was reported by us previously in a scan of just chromosome 1 in an overlapping set of ASPs.14 In HT families from the Sanguenay/Lac St Jean region of Quebec, this locus was linked to HT in obese, but not lean, HTs.29 It therefore appears to house a gene for HT...
FIG. 1. Linkage results across each chromosome by the various tests shown. Markers with LOD >1 are indicated.
of obesity. The locus also shows linkage to familial combined hyperlipidemia and myocardial infarction. The peak marker is at 3.35 Mb and in the region 0 to 13 Mb Ensembl lists 140 unknown and 50 known genes. Of interest are genes for chloride channels (CLCNKA and CLCNKB), natriuretic peptides (NPPA and NPPB), and tumor necrosis factor receptor 2 (TNFRSF1B). Case-control studies have yielded positive findings for CLCNKB and TNFRSF1B in HT. The findings for CLCNKB were moreover supported by functional studies. In contrast, studies of NPPA and CLCNKA have proved negative.

The various genome scans for HT or BP were reviewed in detail recently and inconsistencies in the findings have been tabulated. When investigator claims are made to accord with strict genome-wide significance criteria, many of the significant peaks become suggestive or disappear. The lack of agreement could reflect uneven distribution of alleles due to differences in race or ethnicity between studies, or differences in selection criteria. For example, a locus on chromosome 17 could be for HT of diabetes or obesity. Testing robust intermediate phenotypes has been advocated in trying to overcome heterogeneity of HT etiology.

The largest studies—the National Heart, Lung and Blood Institute (NHLBI) Family Heart Study (1454 white and 1000 African American sibpairs) and the British Investigation of the Genetics of Hypertension (BRIGHT) study in the UK (2010 sibpairs)—yielded results a little better than ours when one excludes a false peak on chromosome 6 in the latter. Moreover, increasing the number of subjects may not be as effective as hoped for due to the underlying genetic heterogeneity. Furthermore, choosing populations with less genetic heterogeneity has not yielded the much hoped for improvement in success. Also, findings to date are consistent with recent suggestions that in HT λ may actually be 1.2 to 1.5.

In conclusion, the GENIHUSS study supports the possible presence of loci for HT on chromosomes 1 and 4, but has yielded little information about the other HT loci expected to be present in the human genome.

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References