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# A Genome-Wide Search For Susceptibility Loci to Human Essential Hypertension

Pankaj Sharma, Jennie Fatibene, Franco Ferraro, Haiyan Jia, Sue Monteith, Chrysothemis Brown, David Clayton, Kevin O'Shaughnessy, Morris J. Brown

**Abstract**—We undertook a systematic search of the entire human genome with the affected sibling-pair model to identify major susceptibility loci to essential hypertension. Affected nuclear families (n=263) were recruited and divided according to definite or probable genetic contribution to hypertension depending on number of hypertensive siblings. The largest nuclear families were first screened with a set of microsatellite markers. Regions on the genome with  $P<0.05$  were tested against the second set of smaller families. An exclusion map was generated to identify regions in which hypertension-causing genes are unlikely to reside. Sibling-pair linkage analysis identified a single locus on chromosome 11q ( $P<0.004$ ) in the first pass. A second pass with nuclear families that had only affected sibling pairs was, as expected, insufficient to support linkage to 11q. Multipoint exclusion-linkage analysis showed that 3 genetic loci are necessary to explain familial aggregation of essential hypertension. Our preliminary findings suggest that no single region within the human genome contains genes with a major contribution to essential hypertension. We show that the disease is indeed polygenic, with each gene providing a relatively small risk. Our exclusion map will help future investigators to concentrate on areas likely to contain these genes. The region on chromosome 11 is the first to point to a new candidate gene for hypertension that has arisen out of a genome search, but replication of these results at a higher significance is necessary before positional cloning can be justified. (*Hypertension*. 2000;35:1291-1296.)

**Key Words:** genes ■ genetics ■ hypertension, essential ■ ROMK

Essential hypertension (EH) is likely to be a polygenic disorder that results from the inheritance of a number of susceptibility genes. Although data from rodent models and human twin-based and population-based epidemiology studies suggest that inherited factors contribute  $\leq 50\%$  of an individual's eventual blood pressure (BP),<sup>1</sup> the number of contributing genes or their individual attributable risk remains unknown. Indeed, whether there are 1 or 2 major hypertension (HT) susceptibility-causing genes with several more minor loci or many genes, each with small attributable risks, is an important question that has not previously been possible to tackle. The affected sibling pair (ASP) model has been useful for identification of loci in various complex traits by determination of how often affected siblings share alleles with each other, given the assumption that diseased alleles are shared more often than predicted by mendelian inheritance.

We have undertaken the first search of the entire human genome for loci linked to EH by use of the ASP model. Our primary objective was to exclude the possibility that EH is due to 1 or 2 major gene effects; our secondary objective was to find some loci with possible evidence of linkage to EH that should be more intensively studied in the larger genome-wide search now under way.

## Methods

### Subjects

Probands were diagnosed as hypertensive if they were already on anti-HT medication. Each proband was sent a questionnaire that inquired about all siblings also on anti-HT medication and sought approval to approach these siblings. Pretreatment BP and current treatment was ascertained for both proband and siblings by their respective general practitioners. Information was also obtained on age, height, weight, drug treatment, histories, and cardiovascular events.

The genome screen was conducted in 2 stages by dividing the nuclear families into 2 independent groups. The first group was enriched for genetic predisposition to HT by use of pedigrees with  $\geq 3$  affected siblings or a proband diagnosed as hypertensive at  $<50$  years of age or with a family history of stroke. An a priori decision was made to replicate in the second independent set of smaller sibships only those regions that produced at a significance of  $P<0.05$  from the initial screen in the 119 families.

### Microsatellite Markers, Polymerase Chain Reaction, and Genotyping

DNA was isolated from whole blood. The standard MRC set of 262 relatively evenly spaced microsatellite markers was used.<sup>2</sup> Polymerase chain reactions (PCRs) contained 50 ng genomic DNA; 1.25 pmol of forward and reverse primers; 50 mmol/L KCl; 10 mmol/L Tris HCL, pH 8.8; 0.1% Triton X-100; 0.2 mmol/L dNTPs; 1.5 mmol/L MgCl<sub>2</sub>; and 0.2 U DyNAzyme II DNA polymerase (Flowgen). Polymerase chain reactions were for 34 cycles at 94°C

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**TABLE 1. Number and Size of Affected Sibling Pairs Used in First Pass of Genome-Wide Screen**

Sibships, n	Actual No. of Sibships	Equivalent No. of Sibships
2	79	79
3	32	64
4	6	18
5	2	8
Total	119	169

Number of affected siblings pairs used in the first pass of the genome-wide screen was based on a conservative estimate of  $K-1$ , where  $K$  is the number of affecteds (eg, a pedigree with 5 affected siblings is the equivalent of 4 (5-1) completely independent ASP).

for 1 minute, 52°C for 1 minute, and 72°C for 45 seconds. Pooled PCR products were precipitated by use of 100  $\mu$ L of 100% isopropanol. One attempt was made to repeat the PCR of samples that initially failed to amplify. Markers that failed to amplify on repeated PCR were discarded. Data from an ABI sequencer was analyzed with Genescan and Genotyper software from ABI (Perkin Elmer) but manually corrected if necessary. Information was analyzed independent of knowledge of pedigree structure.

**Statistical Power of Study**

The number of nuclear families selected for the first pass enabled the present study to be powered to answer our question with the

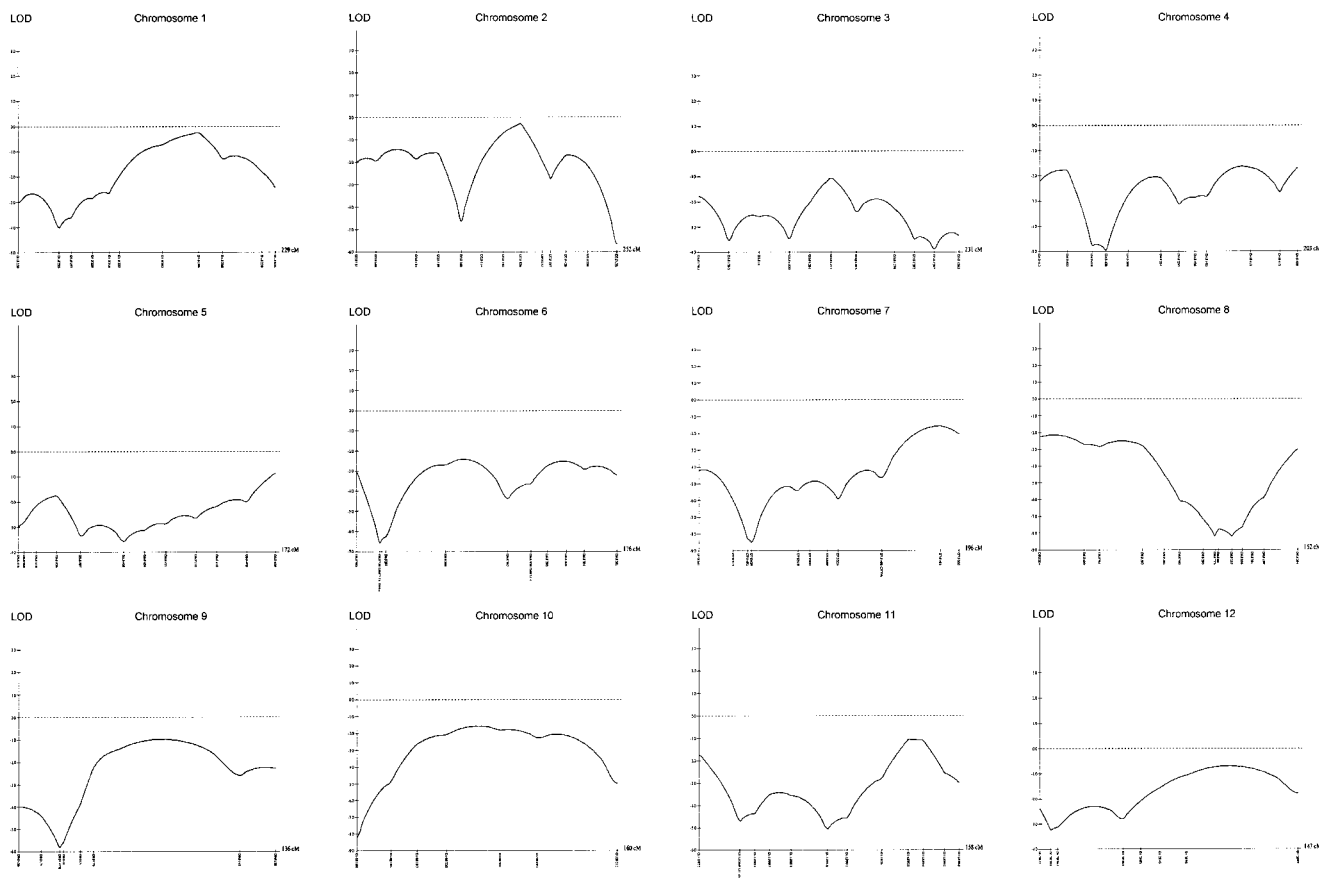
**TABLE 2. Demographic Data on Affected Siblings Used in First Stage of the Genome-Wide Screen**

Demographic Variable	Mean	SD
Gender (M/F), n	102/186	...
Age, y	64.8	10.4
Age at diagnosis, y	52.8	12.0
Body mass index, kg/m <sup>2</sup>	26.3	3.9
Pretreatment SBP, mm Hg	179.6	23.8
Pretreatment DBP, mm Hg	107.7	59.4

expectation of a maximum logarithm of the odds ratio (LOD) score of  $\approx 4.6$ , assuming a small familial clustering ratio,  $\lambda_s$  (a measure of the familial clustering of HT), of 3.5 from our local population.<sup>3</sup> The lower the ratio, the harder it is to find risk genes.

**Statistical Analysis**

Data were analyzed by use of the linkage programs SPLINK, GENEHUNTER, and MAPMAKER/SIBS. All 3 provided nonparametric linkage analyses by use of data on ASPs only, thereby avoiding problems of incomplete penetrance and variable age of onset. Two-point LOD scores were generated by use of SPLINK.<sup>4</sup> Marker allele frequencies estimated by SPLINK were used in GENEHUNTER and MAPMAKER/SIBS. Multipoint analysis was undertaken with GENEHUNTER (version 1.1).<sup>5</sup> MAPMAKER/SIBS was used to generate a genome-wide exclusion map (LOD score threshold, -2.0) to exclude regions unlikely to have a major effect on  $\lambda_s$  of 1.5, 1.8, 2.2, and 2.8, with expected maximum



**Figure 1.** Complete chromosomal results of multipoint analysis with GENEHUNTER. Multipoint linkage maps were generated by use of data from the first 119 nuclear families with allelic frequencies generated internally by SPLINK. All 22 autosomes and the X chromosome are shown.

likelihood scores of 0.9, 1.7, 2.6, and 3.7, respectively. Information regarding recombination distances and phase of markers was obtained as previously published.<sup>2,6</sup>

## Results

Caucasian hypertensive nuclear families ( $n=263$ ) were recruited from the East Anglia region (United Kingdom) with a mean age of 64.5 years (range, 28 to 84), and pretreatment BP range of 260/160 to 180/60 mm Hg. One hundred families, equivalent to 169 conservatively weighted independent ASP (Table 1), met the criteria for the first pass of a full-genome screen. Demographic details of this cohort are shown in (Table 2).

Only 20 regions of the genome were separated by  $>30$  cM, and 9 by  $>35$  cM, which provided an approximate sex-averaged genome resolution of 14 cM with an average marker heterozygosity of  $\approx 0.81$  (SD, 0.06). Complete genome linkage results are presented for each chromosome in Figure 1. One region on 11q met our significance criteria of  $P < 0.05$  (marker D11S934) with 2-point linkage (likelihood  $\chi^2$  score test = 7.9,  $P = 0.004$ ). When the 4 parental chromosomes each have a different allele at a locus, mendelian inheritance should lead to 2 siblings sharing 0, 1, or 2 of these alleles in 25%, 50%, and 25% of sibling pairs, respectively. In contrast, 37% of sibling pairs were identical by descent at marker D11S934.

Multipoint linkage analysis<sup>7</sup> with GENEHUNTER was undertaken on the second set of sibling pairs with 4 markers (D11S910, D11S925, D11S934, and D11S968) and 3 addi-

tional markers (D11S975, D11S4463, and D11S933) in only the 11q region. As was likely from the number of sibships available in the second set and their presumed lower genetic contribution to HT, GENEHUNTER did not detect any significant excess of allele sharing above that expected by chance alone (0.25, 0.5, and 0.25) in this set for the markers studied.

## Exclusion Mapping

The genome-wide exclusion map shows that  $\approx 92\%$  of the human genome can be excluded because they exhibit  $\lambda_s$  of 2.8 and  $\approx 71\%$  because they exhibit  $\lambda_s$  of 1.8 (Table 3), which demonstrates that no 1, and probably no 2, regions alone account for the overall  $\lambda_s$  of 3.5 in our population of EH. Only the complete chromosome exclusion map for chromosome 11 is shown graphically (Figure 2).

## Discussion

HT affects  $\approx 25\%$  of adults and is a major cause of cardiovascular disease. Despite a half-century of research, HT remains quaintly "essential" in  $>90\%$  of patients. Identification of its genetic basis is currently our best hope for making progress in understanding of its origin. We report the first general systematic genome-wide search for major susceptibility loci to EH. Genome-wide searches have been used to identify loci for a variety of common diseases by use of the ASP model, which seeks to determine the frequency of allele sharing among affected siblings. Assumptions about the

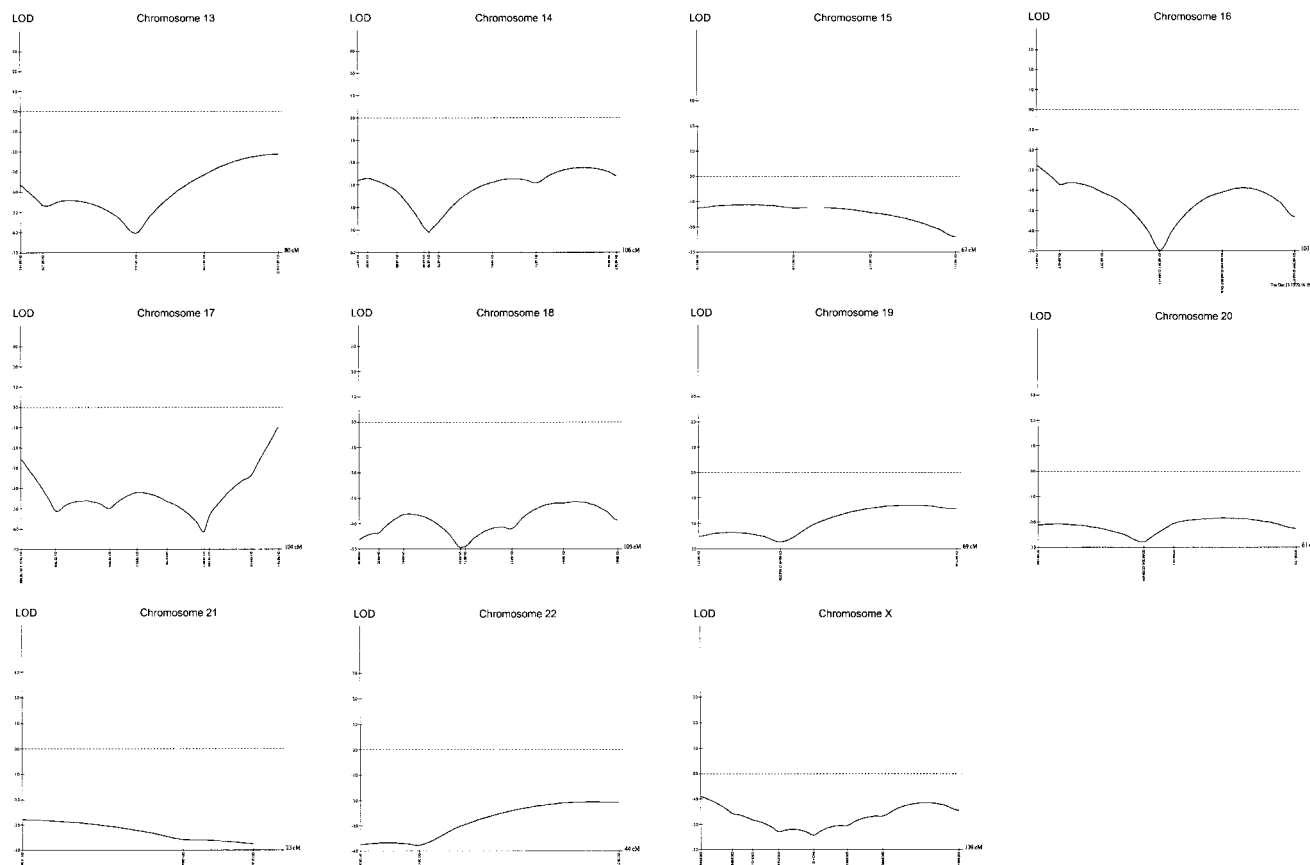


Figure 1. Continued.

TABLE 3. Genome-Wide Exclusion Map

Chromosome	$\lambda$			
	1.5	1.8	2.2	2.8
1	50	64	86	100
2	6	42	67	94
3	60	90	100	100
4	49	97	100	100
5	15	77	87	100
6	35	69	92	100
7	80	100	100	100
8	47	79	100	100
9	50	50	50	60
10	35	82	100	100
11	59	85	100	100
12	39	64	73	85
13	82	100	100	100
14	13	60	87	100
15	0	23	50	85
16	100	100	100	100
17	77	100	100	100
18	57	100	100	100
19	0	19	50	62
20	0	44	72	100
21	0	100	100	100
22	45	82	100	100
X	0	0	8	24
Mean% genome excluded	39	71	84	92

MAPMAKER/SIBS was used to assess the percentage of each chromosome excluded on the basis of different contributions to the  $\lambda$  ratio (maximum possible, 3.5) of essential hypertension (see text).

mode of inheritance of disease are not required, but this model does generate the need to recruit many siblings. Although EH is now the most prevalent of the common diseases studied to date with this model, the recruitment of enough affected siblings to answer the question of the existence of major loci still took  $>2$  years.

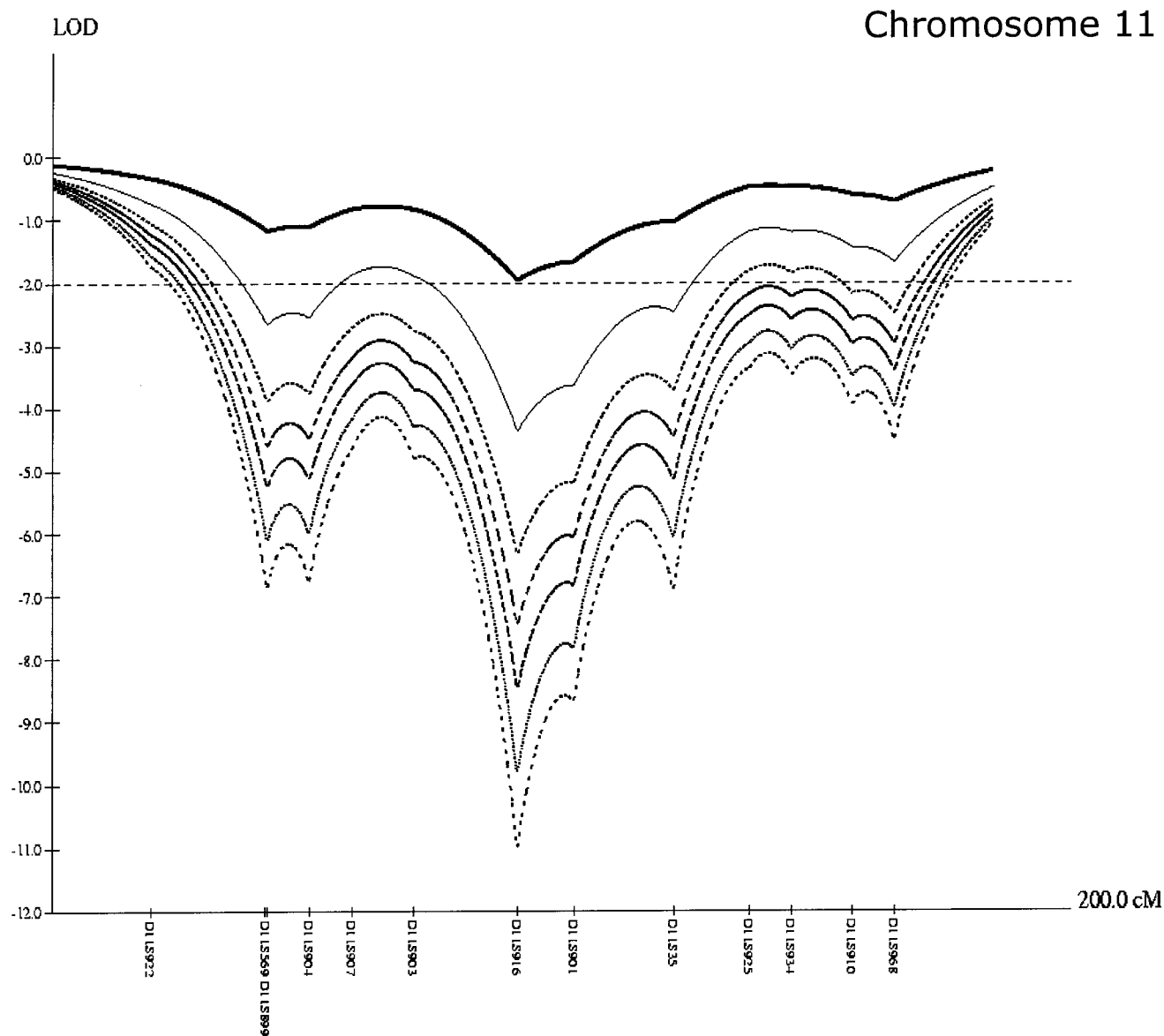
Our genome search used a 2-stage strategy. The first stage involved use of only multiple (trios or more) affected sibships to maximize the likely inherited component to BP. A region on 11q was highlighted with 2-point linkage. However, this result was obtained without correcting for multiple testing. If one were to correct for all markers used, then the 2-point result would not be significant, but such a Bonferroni correction assumes that all markers are acting independently, which they are not. Indeed, the exact statistical measures to apply are rigorously contested.<sup>8-10</sup> Notwithstanding these controversial issues, our exclusion-map data for the first time demonstrates (1) that no one region (not even 11q or chromosome 1, which contains the controversial angiotensinogen locus<sup>11,12</sup> provides a major ( $\lambda_s \geq 2.8$ ) contribution to the heritability of EH; (2) that at least 3 HT genes exist,<sup>3</sup> each with a small attributable risk; and (3) that EH is a polygenic trait. Indeed, many of the chromosomes are excluded from

containing major HT risk loci (Table 3), with the possible exceptions of chromosomes 2, 9, 12, 15, 19, and the sex chromosome. Such exclusion mapping can be useful with ASP analyses in complex disorders, because penetrance is not an issue. The other main criticism of exclusion mapping concerns the heterogeneity of the disease process. Although this could be an issue in HT, our geographic region is known to contain a stable and well-characterized hypertensive population, which considerably reduces but does not entirely exclude this problem.

Although a second, smaller independent cohort of ASP was unable to support linkage on chromosome 11q, failure to replicate does not exclude that region, because the number of families known to be required for replication is approximately  $n-1$  times as many as initially required to demonstrate linkage (where  $n$  is the number of loci involved).<sup>13</sup> Thus, if EH is regulated by, for example, a half-dozen clinically important genes, as many as  $6 \times 169 = 1014$  families may be needed, whereas our second set consisted of 144 families. Thus, our 2-part strategy effectively demonstrates the previous theoretical predictions that reproducibility is likely to require much larger numbers of siblings (probably in the thousands) than the original observations. This is even more so if the genetic liability threshold is greater in nuclear families with multiple affected siblings (first pass) compared with those of only ASPs (second pass). Failure to replicate could be due to an initial false-positive result at 11q. Therefore, our results, although interesting, should be viewed as no more than a first step toward identification of regions that contain hypertensive loci. The "gaps" in our genome map may contain genes of importance and need to be concentrated on in future studies.

These results lead to the question of whether the present study was adequately powered. The 169 independent sibling pairs used in the present study were sufficient to detect a single susceptibility locus (if one existed) with a maximum LOD score of 4.6. Clearly, with a larger number of sibships and a denser map, genes with smaller attributable risk can be located. Such large studies are underway but are unlikely to report for another 2 to 3 years. Even these could miss all but the most important loci, whereas small screens can by luck identify loci of modest importance (LOD scores  $<2$ ).<sup>14</sup> Clearly, the need for many smaller genome scans to report efforts to identify major susceptibility loci will help ongoing studies to focus quickly on interesting chromosomal regions. Notwithstanding this caveat, our results are consistent with 2 recent reports<sup>15,16</sup> that attempt to identify BP loci by use of the discordant sibling model,<sup>17</sup> which has pointed to a number of potential (and presumably low-attributable-risk) loci throughout the human genome.

The genomic region highlighted on chromosome 11q with 2-point linkage contains the ROMK gene, which is known to be causative in Bartter's syndrome, a monogenic BP-influencing disorder.<sup>18</sup> The chance of this level of a hit occurring over a chromosomal region that contains 1 of the 8<sup>19-26</sup> known monogenic BP-influencing disorders was investigated under a multiple-test scenario, and the result remains significant at the 5% level ( $P=0.03$ ). However, even if this result is replicated by other studies, it may be premature to



**Figure 2.** Chromosome 11 exclusion map generated by use of MAPMAKER/SIBS with recurrence risks,  $\lambda_s$ , of 1.2 (lowest dotted line), 1.5, 1.8, 2, 2.5, and 2.8 (solid line), respectively. LOD threshold of  $-2$  was used throughout.

suggest ROMK as a hypertensive gene, because the region of positive linkage spans  $\approx 62$  cM and potentially contains  $\approx 2000$  genes.

In conclusion, our preliminary systematic search of the entire human genome for major susceptibility genes for EH demonstrates that no single region makes a large contribution to its origin. We demonstrate that HT is likely to be accounted for by several genes and that high BP is indeed a multigenic trait. The exclusion map generated from this work will be an important tool to focus future much larger studies on regions likely to contain genes with more modest effects.

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