Genome-wide linkage reveals a locus for human essential (primary) hypertension on chromosome 12p

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Essential (primary) hypertension is an important risk factor for cardiovascular morbidity and mortality. Blood pressure is largely heritable; however, the genetic factors contributing to essential hypertension are mostly unknown. We examined a large Chinese kindred ($n = 387$) and selected a subset of 94 individuals for genotyping. An additional 32 Chinese nuclear families with essential hypertension were also recruited. Genome-wide parametric linkage analysis identified a new locus for primary hypertension on chromosome 12p (parametric LOD score 3.44). This locus overlaps with the assigned locus that causes severe autosomal-dominant hypertension and brachydactyly, the only form of monogenic hypertension known to date that resembles primary hypertension. We suggest that this genomic region, spanning 18 annotated genes, will be of great relevance in elucidating new mechanisms for primary hypertension.

INTRODUCTION

Death and disability from cardiovascular disease is increasing rapidly in all countries so that cardiovascular disease will still be the commonest cause of death worldwide early in this century (1,2). Arterial hypertension is a major risk factor for stroke and myocardial infarction (3). Although hypertension may be secondary, most (>95%) hypertension is ‘essential’ or primary. Primary hypertension, which affects about 20% of adults, is partly genetically determined; the estimates on genetic variance range from 20 to 50% (4–7).

Epidemiological data suggest that there is a remarkable difference in the level of blood pressure and the geographic regions in China with a prevalence of hypertension ranging from as low as 3% to more than 20% (8). Comparison of epidemiological blood pressure data is difficult due to subtle but important differences in the circumstances and techniques used for blood pressure measurements. However, there is epidemiological evidence that the age-specific prevalence of hypertension in China is lower compared with westernized countries but similar to other developing countries (8–10). The prevalence of secondary hypertension in Chinese communities was reported to be slightly higher than 1% (11).

Primary hypertension is extremely heterogeneous and the genetic contributions remain largely unknown (12). Several studies have investigated linkage and/or association of candidate genes in primary hypertension but most do not appear to contribute to primary hypertension. Only two contribution of two candidates—the angiotensinogen gene on chromosome 1 and the alpha adducin gene on chromosome 4—have held up in multiple studies (13). There have been several genome-wide linkage scans of familiar blood pressure

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distribution/variation (14–20) and for essential hypertension (21–24), most of them not attaining significance at a genome-wide level. One possible approach to the problem of the tremendous genetic heterogeneity associated with primary hypertension is the ascertainment of hypertensive pedigrees from relatively isolated populations. We pursued such a strategy and recruited hypertensive pedigrees from a relatively isolated rural region in China (Table 1). Here, we report significant linkage to chromosome 12p in our genome-wide linkage study in a large Chinese kindred and additional nuclear families.

Table 1. Basic phenotype characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Large kindred</th>
<th>Nuclear families</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Affected</td>
<td>Unaffected</td>
</tr>
<tr>
<td>Number</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>43.7</td>
<td>47.8</td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>48.7 ± 10.6</td>
<td>46.3 ± 13.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg, mean ± SD)</td>
<td>98.3 ± 11.3</td>
<td>78.0 ± 17.4</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg, mean ± SD)</td>
<td>154.8 ± 7.8</td>
<td>122.6 ± 14.3</td>
</tr>
<tr>
<td>Antihypertensive therapy (%)</td>
<td>37.8</td>
<td>—</td>
</tr>
</tbody>
</table>

RESULTS

A maximum multipoint LOD score of 2.82 was obtained at marker D12S1682. With the 16 additional markers, a multipoint linkage analysis revealed a maximum parametric LOD score of 2.85. To expand on these findings, we recruited 32 nuclear families (parents and offspring) with essential hypertension, $n = 5.4$ per family totaling 174 individuals from the Shijingshan district. Parametric linkage analysis revealed a significant effect of chromosome 12 on blood pressure when the two subsets were combined (parametric LOD score 3.44; Fig. 1). These findings strongly support the presence of a major hypertension gene within this chromosomal segment.

All individuals classified as hypertensive consistently had elevated systolic and diastolic blood pressure levels. Thus, no reclassification according to systolic or diastolic blood pressure was necessary. The observed linkage on chromosome 12p holds for both systolic and diastolic blood pressure levels.

We were surprised to find a nearly perfect overlap with a previously identified monogenic high blood pressure locus detected in a Turkish family with severe autosomal hypertension and brachydactyly as shown in Figure 2 (25–27). This monogenic hypertension resembles primary hypertension since the renin-angiotensin system is normal and affected persons are not salt-sensitive (28). None of our subjects have brachydactyly. However, the syndrome in the Turkish subjects may be a contiguous gene syndrome and the brachydactyly may not necessarily be caused by the same gene generating the increased blood pressure.
**DISCUSSION**

Possibly, ‘less dramatic’ molecular variants of genes causing severe forms of monogenic hypertension contribute to the far more common, primary hypertension. However, evidence to support this notion is scanty. Our findings demonstrating linkage to primary hypertension in Chinese to chromosome 12p, coupled with the fact that severe autosomal-dominant hypertension in the Turkish kindred resembles primary hypertension, strongly suggests that the identification of responsible gene(s) within this genomic region will be generally relevant. The locus has been cloned and sequenced in its entirety (29,30) providing a list of annotated positional candidate genes across this region. Furthermore, single-nucleotide polymorphisms (SNPs) are available for systematic approaches to define the disease-associated mutation(s) (see Ensembl and Celera databases at www.ensembl.org and www.celera.com).

Two candidate genes reside on chromosome 12p that may play an important role in the maintenance of vascular function and blood pressure. *PDE3A* is a cyclic nucleotide phosphodiesterase whose physiological role is the attenuation of the signaling mediated by the ubiquitous second messangers cAMP and cGMP (31). *PDE3A* and *PDE3B* both interact with the renin–angiotensin system. The renin–angiotensin system is important in controlling blood pressure, maintaining electrolyte and volume homeostasis, and regulating renal autoregulation (32). Another candidate of equal interest encodes the sulfonylurea receptor *ABCC9* (SUR2). Sulfonylurea receptors represent one of four subunits in the assembly of ATP-sensitive potassium channels. The activity of this channel can be altered by a diverse group of drugs, so-called potassium channel openers. These drugs may have utility in lowering blood pressure (33).

Linkage was not detected in any other region of the genome. This might have been due to the limited power of our study, which was determined to be 58.7 and 20.4% for suggestive and significant linkage, respectively. It may be that the current cohort was not sufficiently large to identify additional or previously reported loci of small or moderate effect.

We provide strong evidence for a susceptibility locus on chromosome 12p. Although the locus was identified in Chinese

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**Figure 2.** Representation of the region linked to essential hypertension in this report and the previously reported linkage to hypertension in a Turkish family with brachydactyly (21–23).
families from a relatively isolated rural region, the overlap with a Mendelian form of hypertension with brachydactyly in a Turkish kindred suggests that lesser variants of the same gene might contribute to primary hypertension.

The data presented here make this genomic region amenable for dense association scans in positional candidates. We suggest that susceptibility gene(s) for essential hypertension may reside on chromosome 12p.

METHODS

We ascertained an extended pedigree with information from 387 members over seven generations from a rural village from Shijingshan, China (34). Hypertension occurs in 24%. Blood pressure measurements from all family members were obtained several times over a 2–5 year period. Blood pressure was measured with a mercury sphygmomanometer in seated subjects after 5 min rest. Three measurements were obtained 1 min apart and the values averaged. Subjects with values >140/90 mmHg (below age 35 years) or >160/95 mmHg (above age 35 years) were defined as hypertensive. Individuals prescribed antihypertensive drugs were also defined as hypertensive (Table 1). However, since longitudinal blood pressure measurements were ascertained, extensive and unambiguous documentation of pretreatment hypertensive values were available. None of the individuals while being treated with antihypertensive drugs had normotensive blood pressure values below 140/90 mmHg.

Ten adult hypertensive family members from both genders aged 34–75 were randomly selected from the large pedigree. These persons underwent extensive clinical investigations to exclude any causes of secondary or known monogenic forms of hypertension (12) (data not shown). Ninety-four family members were selected according their availability for genetic analysis (48 affected and 46 non-affected). Additionally we recruited 32 nuclear families (parents and offspring with essential hypertension totaling 174 individuals; n = 5.4 per family), according to the same criteria as described above. After informed consent, blood was obtained for DNA extraction. Again 10 hypertensive individuals were randomly selected to exclude secondary hypertension.

A genome-wide linkage analysis using 387 polymorphic microsatellite markers was performed. We carried out fluorescence-based semi-automated genotyping using 387 autosomal microsatellite markers from the Génétôn linkage map (35) with an average heterozygosity of 0.8. We typed 16 additional markers on chromosome 12 where linkage was detected. After individual PCR amplification, products were pooled and size-fractionated by electrophoresis on MegaBase 1000 (Amersham Pharmacia) or ABI 3100 (Applied Biosystems) DNA capillary sequencers.

Individual markers were completely typed on one genotyping station. We determined allele sizes using the GeneticProfiler (Amersham Pharmacia) or Genescan 2.1.1. and Genotyper V2 software (Applied Biosystems). All marker genotypes were checked for Mendelian inheritance using the PedCheck software (36). Parametric linkage analysis was performed with Genehunter V2.1.3 (37) using marker distances according to the Génétôn linkage map (35) and uniform allele frequencies. A dominant (0.02, 0.7, 0.7) inheritance model and a disease allele frequency of 0.14 were assumed. For linkage analysis, we divided the entire large pedigree down to eight smaller branches because of computational constraints. Statistical power calculations were performed with Allegro (38) simulating 1000 genome-wide replicates with the same specifications that were used in the subsequent final analysis. The power of this study was determined to be 58.7 and 20.4% to detect suggestive and significant linkage, respectively.

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