Blood pressure QTLs identified by genome-wide linkage analysis and dependence on associated phenotypes

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Harrap, Stephen B., Zilla Y. H. Wong, Margaret Stebbing, Angela Lamantia, and Melanie Bahlo. Blood pressure QTLs identified by genome-wide linkage analysis and dependence on associated phenotypes. Physiol Genomics 8: 99–105, 2002. First published December 4, 2001; 10.1152/physiolgenomics.00069.2001.—Understanding genetic factors that contribute to population-wide variation in blood pressure is likely to benefit prevention and treatment of cardiovascular disease. The aim of the Victorian Family Heart Study is to identify genes for cardiovascular risk in 783 volunteer adult families recruited from the general population. In this preliminary study we sought to identify quantitative trait loci (QTLs) using a genome-wide linkage analysis in 274 adult sibling pairs of average age 24 yr selected without respect to blood pressure. We compared multipoint linkage results for carefully measured systolic (SBP) and diastolic (DBP) pressures before and after statistical adjustment for covariation with sex, oral contraception, age, height, and weight. The average BP was 123/67 (SD: 12/11) mmHg in males (n = 283) and 114/64 (SD: 10/9) mmHg in females (n = 265). Nonparametric Z-scores from multipoint GeneHunter II analysis were “suggestive” (3.1 or more) at four QTLs for SBP (chromosomes 1, 4, 16, and X) but at no QTLs for DBP. Most Z-scores were affected little by adjustment for covariates. However, the SBP QTL on chromosome 16 was obvious only for unadjusted pressures. This population-based quantitative trait analysis has identified more QTLs than any of the eight previous genome-wide scans for blood pressure. Considerable discrepancies between different studies may reflect the presence of false-positive results or real biological differences between populations.

A MOLECULAR UNDERSTANDING of the genetic basis of variation in blood pressure is relevant to the development of new strategies to prevent and treat high blood pressure and related cardiovascular complications. Genome scans offer a comprehensive search for genetic loci that may be worthy of further detailed analyses for gene identification and mutation detection. In this study we present the results of a preliminary genome-wide search for blood pressure quantitative trait loci (QTLs) in white families drawn from the general population and report the position of four QTLs for systolic blood pressure. These QTLs span a number of important candidate genes. We also report that evidence in favor of linkage at one systolic blood pressure (SBP) QTL disappeared after adjustment for sex and body size. Finally, we compare the results of published genome scans and consider their similarities and differences.

MATERIALS AND METHODS

This study is a core component of the Victorian Family Heart Study (VFHS). The details of the recruitment and phenotyping in the VFHS have been described in detail elsewhere (7). In brief, the aim of the VFHS was to recruit white families that represent the population range of cardiovascular risk phenotypes including blood pressure. Recruitment was population based, and the cardiovascular risk phenotypes of our subjects were typical of the general Australian community (7). A family history of heart disease was not relevant to recruitment. Families comprised, at a minimum, a mother and father with at least one natural child. A family was eligible if both parents were aged between 40 and 70 yr and at least one offspring was aged between 18 and 30 yr. Other offspring were also included if they were aged between 18 and 30 yr. The lower age threshold for offspring was set to minimize the confounding effects of growth on the phenotypes under study. A total of 783 families, comprising a total of 2,959 individuals, were recruited between 1991 and 1996 (7).

Selection of sibling pairs. The aim of this analysis was to screen the genome for QTLs that could be studied in greater detail in larger numbers of families at a later stage. For this first-pass screen we selected a total of 275 sibling pairs. These pairs were selected at random from recruited families that had two or more children. Only one pair was recruited from each family, assuring independence of pairs (2). Known monozygotic twins or twins of the same sex with uncertain zygosity were excluded from the linkage analyses. Following the linkage analysis, error checking (see below) revealed that one pair was in fact monozygotic twins. This pair was excluded, and the data reported here refers only to the remaining 274 sibling pairs.

Phenotype measurement. These studies were approved by the Ethics Review Committee of the Alfred Hospital, Melbourne, and informed consent was obtained from all participants. Participants attended one of our study research clinics between 9 AM and 7 PM. Weight and height were measured after removing heavy clothing and shoes. Subjects then...
rested supine for 10 min, during which time a suitably sized sphygmonanometer cuff was applied to the right arm. Blood pressures were measured by carefully trained research nurses using a standard mercury sphygmonanometer and were subject to routine quality control checks. SBP was taken at the return of arterial sounds (Korotkoff phase I) and diastolic blood pressure (DBP) at the disappearance of sounds (Korotkoff phase V). Blood pressure measurements were made to the nearest 2 mmHg. Three measurements of SBP and DBP were taken. The first reading was discarded to avoid potential bias, and the last two were recorded and averaged to give values for SBP and DBP used in these analyses. Detailed information was obtained regarding treatment with oral contraceptive or hormone replacement therapy, antihypertensive medications, and lipid-lowering therapy. Following phenotypic measurements, venous blood was collected for DNA extraction.

Phenotype standardization and adjustment. The primary linkage analysis was planned for raw blood pressures in the 274 sibling pairs of the offspring generation. A secondary linkage analysis was planned to utilize blood pressure phenotypes that had been standardized and adjusted for the effects of important covariates associated with blood pressure in this group. To obtain the most representative estimates of these covariate effects, we used data from the entire group of 1,410 offspring. Both SBP and DBP were significantly different in males (n = 674, 122/67 mmHg) and females (n = 736, 114/65, P < 0.0001) and between women taking (n = 320, 116/66 mmHg) or not taking (n = 416, 112/64 mmHg, P < 0.0001) oral contraception.

Therefore, SBP and DBP data were initially standardized as Z-scores (SBPZ and DBPZ, respectively, calculated by dividing the difference between each individual value and the group mean by the standard deviation for the group) within each of three separate groups: men, women taking oral contraception, and women not taking oral contraception. The only exceptions to this standardization procedure were subjects taking antihypertensive medications. In such cases (5 of the 1,410 total offspring) we assumed that the recorded pressures were not necessarily reliable indicators of untreated pressures and would otherwise be expected to be in the upper 10% of the distribution. Therefore, as in previous studies (21) where SBPZ and DBPZ on treatment fell below the 90th percentile (Z < 1.64) the value of 1.64 was substituted. This situation applied to only one female in the sibling pair analysis (see below).

SBPZ and DBPZ from the three groups were combined for further adjustment. Age correlated with DBPZ (n = 1,410, Spearman r = 0.20, P < 0.0001), and DBPZ was adjusted by regression on age. Finally, sex-standardized values of weight were standardized by the Australian population (7). The phenotypes of the selected sibling pairs resembled closely those of the complete set of VFHS offspring. Figure 1 shows the distribution

RESULTS

Subject characteristics. Table 1 presents the basic characteristics of the 548 individuals who comprised the 274 sibling pairs and compares them with all 1,410 offspring in the VFHS. As reported previously, the VFHS subjects are representative of the general Australian population (7). The phenotypes of the selected sibling pairs resembled closely those of the complete set of VFHS offspring. Figure 1 shows the distribution
of measured SBP (Fig. 1A) and DBP (Fig. 1B) for the 548 individuals. Of the 548 subjects, two women were being treated for hypertension. Blood pressure in one was below the 90th percentile, and she was given SBPZ and DBPZ of 1.64 (see MATERIALS AND METHODS).

The sex distribution was relatively even, with 283 men and 265 women. Within the pairs there was a relatively even balance of the sexes, with 65 male-male, 135 male-female, and 74 female-female pairs. The distributions of the absolute values for SBP and DBP differences between sibling pairs are shown in Fig. 1, C and D, respectively. The median for the absolute difference in SBP pressure between siblings was 10 mmHg with an interquartile range of 4–16 mmHg. For absolute differences in DBP between siblings, the median value was 8 mmHg and the interquartile range was 4–13 mmHg.

**Genome linkage.** Figures 2 and 3 show the probability plots of nonparametric Z-score values for linkage of SBP and DBP, respectively, across all 22 autosomes and the X chromosome. Figures 2 and 3 show data from linkage analyses of raw (thick line) and adjusted (thin line) pressure phenotypes. In general, the Z-scores for these phenotypes describe similar curves, with some exceptions (see below). In this first-pass analysis, we identified suggestive QTLs as those with Z-scores of 3.1 or greater according to the guidelines of Lander and Kruglyak (13). Such values were observed for four loci associated with SBP (see Table 2 and Fig. 2).

For SBP a Z-score peak of 3.5 on chromosome 1 spanned the markers D1S255, D1S2797, D1S2890, and D1S230. Another peak on chromosome 4 (Z = 3.2) spanned markers D4S1534, D4S414, D4S1572, D4S406, D4S402, and D4S1575. On chromosome 16 in a region that spans the markers D16S3046, D16S3068, and D16S3136, a peak Z-score of 3.2 was seen for

<table>
<thead>
<tr>
<th>Table 1. Relevant phenotypes in the Victorian Family Heart Study in men and women of all 1,410 offspring and the 548 offspring included as the 274 sibling pairs in the genome scan</th>
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<tbody>
<tr>
<td><strong>All Offspring</strong></td>
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<td></td>
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<tr>
<td><em>n</em></td>
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<tr>
<td>Age, yr</td>
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<tr>
<td>Height, cm</td>
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<tr>
<td>Weight, kg</td>
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<tr>
<td>DBP, mmHg</td>
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<td>SBP, mmHg</td>
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Values are means; SD are in parentheses. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Fig. 1. Frequency distributions from 548 individuals comprising 274 sibling pairs of individual systolic blood pressures (SBP, mmHg; A), individual diastolic blood pressures (DBP, mmHg; B), absolute differences in systolic pressure (mmHg) between siblings (C), and absolute differences in diastolic pressure (mmHg) between siblings (D).
DISCUSSION

We undertook a preliminary genome-wide scan in search of chromosomal QTLs linked with population variation in blood pressure and to determine whether linkage results were influenced by important covariates. Based on evidence of “suggestive” genome-wide probabilities, this first-pass genome investigation located four QTLs that warrant closer attention, one of which showed evidence of dependence on blood pressure covariates. The relevant QTLs were observed on chromosomes 1, 4, 16, and X.

The QTL on chromosome 1 (1p34.3-1p31) may be syntenic to a region on rat chromosome 5 that has been linked with blood pressure (4). The human candidates in this region include genes encoding the leptin receptor (LEPR) and the transforming growth factor-β type III receptor (TGFBR3). It is noteworthy that the QTL on chromosome 1 does not include the angiotensinogen gene (AGT) at 1q42-q43, which has been suggested as relevant to hypertension (10). The observed QTL on chromosomes 4 (4q21-4q28) may be syntenic to blood pressure locus on rat chromosomes 2 (3), but no outstanding human candidate genes are known presently in this region.

The QTL on chromosome 16 (16p13.1-16p12) is consistent with our previous linkage results with a separate set of markers around the epithelial sodium channel β- and γ-subunit genes (SCNN1B and SCNN1G) in this region (22). The SCNN1B and SCNN1G genes have been implicated in the etiology of Mendelian diseases associated with very high or very low blood pressures (16). However, there are also other relevant candidate genes in this region, including the genes encoding a norepinephrine transporter (SLC6A2) and the renal thiazide-sensitive NaCl transporter (SLC12A3) and the renal sodium-glucose cotransporter (SLC5A2).

The SBP QTL on the X chromosome (Xp11.4-Xq11) coincides with a region that encompasses the candidate genes encoding the androgen receptor (AR), monoamine oxidase A (MAOA), monoamine oxidase B (MAOB), and the renal voltage-gated chloride channel.

Fig. 2. Z-score values for genome-wide scan of linkage for systolic blood pressure levels. Thick black lines represent raw pressure levels, thin black lines represent adjusted pressure levels (see text for details).
The X chromosome is the second human sex chromosome to be associated with blood pressure variation. We recently reported significant association of diastolic pressure with a polymorphism in the nonrecombining region of the Y chromosome (5). Interestingly, both the X and Y chromosomes have been linked with significant blood pressure variation in spontaneously hypertensive rats of the stroke-resistant and stroke-prone strains (6, 8).

Recently a number of genome scans concerned with blood pressure have been reported (9, 12, 15, 18–20, 23, 24). Table 2 summarizes these studies by listing the QTLs that satisfy the genome-wide “suggestive” significance level, taken as a Z-score of 3.1 or greater, a log of the odds ratio (LOD) score of 2.2 or greater, or a $P$ value of 0.0007 or less (13). The comparison of studies reveals a number of interesting features. Most studies have reported linkage with SBP rather than DBP. There is no obvious explanation for the lack of evidence of DBP QTLs.

The number of QTLs detected varies between studies from zero QTLs in two studies in hypertensive sibling pairs (18, 20), to four QTLs in the present study. Issues of statistical power are of potential relevance here.

Table 2. Summarized multipoint linkage results of genome scans for blood pressure

<table>
<thead>
<tr>
<th>No of QTLs</th>
<th>Chromosomes</th>
<th>Phenotype</th>
<th>Ethnicity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>4</td>
<td>1, 4, 16*, X</td>
<td>SBP</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Rice et al.</td>
<td>3</td>
<td>2, 5, 7</td>
<td>SBP</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Xu et al.</td>
<td>2</td>
<td>15, 16*</td>
<td>DBP, SBP</td>
<td>Chinese</td>
</tr>
<tr>
<td>Krushkal et al.</td>
<td>1</td>
<td>6</td>
<td>SBP</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Levy et al.</td>
<td>1</td>
<td>17</td>
<td>SBP</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Zhu et al.</td>
<td>1</td>
<td>2</td>
<td>SBP</td>
<td>Chinese</td>
</tr>
<tr>
<td>Hseuh et al.</td>
<td>1</td>
<td>2</td>
<td>DBP</td>
<td>Caucasian</td>
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<tr>
<td>Perola et al.</td>
<td>0</td>
<td></td>
<td>ITT</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Sharma et al.</td>
<td>0</td>
<td></td>
<td>ITT</td>
<td>Caucasian</td>
</tr>
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</table>

The number of quantitative trait loci (QTLs) given represents those for which the significance of linkage exceeded the genome-wide “suggestive” criteria of Lander and Kruglyak (13). HT, hypertension. * Possible overlap of the QTLs on chromosome 16 in these two studies.
Power may be limited by relatively small numbers of subjects. However, even some of the larger studies found only one QTL (15).

The discrepancies in the locations of the QTLs between reported studies are striking. At only one QTL on chromosome 16 (present study and Ref. 23) does there appear to be any suggestion of similarity. There are a number of possible explanations for such inconsistency. One potential explanation is the use of different panels of map markers between the different scans. Another explanation is statistical. The level of statistical significance attributable to QTLs that satisfy “suggestive” linkage leads one to expect one false-positive QTL per genome-wide scan (13). Only the present study and two of the previously published studies (19, 23) report more than one QTL at this level of significance. One study (15) reported a single QTL with a probability level well beyond the “suggestive” threshold. If one assumes the presence of one false-positive per genome scan at this level of significance, then it is not surprising that many of reported QTLs do not coincide, particularly where limited numbers of QTLs are detected in individual studies. There may be other biological explanations for the incongruities between reported QTLs, such as underlying genetic and environmental variation between the populations studied. Should this prove to be the case, it raises doubts regarding the general applicability of individual QTLs that are not reproducible in different populations. The exact nature of the phenotype may also be important. The different linkage patterns of DBP and SBP may be a relevant example of the specificity of blood pressure phenotype. It is not certain whether more subtle differences, such as the posture in which blood pressures are measured, are important. In the published studies, blood pressures have been measured in the sitting position (9, 15, 24), but sometimes posture was unspecified (12, 19, 20, 23).

In some studies covariates of blood pressure such as body size were taken into account (9, 15, 19); in others they were not. It appears from the findings in the present study that at some QTLs blood pressure covariates may have important effects on linkage results and potentially explain study discrepancies. For example, if sex and body size influence blood pressure independent of a particular blood pressure QTL, failure to adjust for their effects will blur linkage detection. We did not find substantial evidence of such an effect at any QTL. However, we found the reverse situation in relation to the chromosome 16 QTL at which the Z-score fell from 3.2 for unadjusted SBP to 1.4 for adjusted SBP. Such an effect might be explained if sex and body size interact cooperatively and specifically with genetic mechanisms encoded by the particular QTL. For example, the blood pressure effect of a QTL might be enhanced in the presence of increased body mass index, or a QTL might increase both blood pressure and body mass index. Under these circumstances, the adjustment of blood pressure for covariation in body mass index would diminish residual blood pressure variation attributable to such a QTL and limit the apparent strength of linkage. Finally, in light of the strong influence of growth on blood pressure in adolescence (14), linkage analyses that include younger subjects might reveal growth rather than blood pressure QTLs unless correction is made for growth effects.

In summary, this genome scan reveals four QTLs for SBP and would, therefore, be consistent with the presumed polygenic nature of blood pressure variation. No QTLs for DBP were detected. We also found that the linkage probability of one QTL seemed to depend on association with sex and body size. Further multivariate analyses are required to clarify this possibility. Our study shows that it is possible to detect QTLs in sibling pairs selected at random from a representative population sample. Our deliberate epidemiological approach to sampling and recruitment is intended to identify QTLs relevant to population-wide variation in blood pressure. These QTLs are relevant not only to high individual cardiovascular risk (highest pressures) but also high population-attributable cardiovascular risk (average to high pressures).

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REFERENCES


