Hypertension is a common and complex disorder that affects 31.0% of people aged 45 years and 77.6% of subjects aged 75 years, thereby substantially increasing their risk for cardiovascular disorders, notably ischemic heart failure and stroke. Numerous studies have shown that genetic factors play a role in hypertension, as well as in the underlying quantitative trait, blood pressure (BP). The heritability for both systolic BP (SBP) and diastolic BP (DBP) is approximately 50% and is remarkably stable over populations and time, but the identification of quantitative trait loci (QTL) influencing BP and hypertension is a complex endeavor. Many genome scans have been performed, but most of the reported BP loci are not yet robustly replicated, and larger studies and meta-analyses of data tend to show less significant results. Reasons for these findings may include a large number of genes with small effects involved, which are difficult to detect; true heterogeneity between populations; the sometimes relatively small sample sizes; different designs and analytical approaches; different definitions of the phenotype (hypertension or quantitative BP scores); different treatment of subjects who take antihypertensive medication; and the use of different covariates.

In 2 recent studies from the Netherlands Twin Register and the Australian Twin Register, we found that the genetic variance in longitudinal BP data is largely attributable to stable genetic factors. Simulations have shown that the use of such longitudinal data sets increases the power to detect QTLs. We carried out a scan on repeated measures of BP over a 7- to 12-year period to localize genes that may explain BP heritability.

Methods

Subjects

Longitudinal BP data for 3472 monozygotic (MZ) twins, dizygotic (DZ) twins, and siblings were collected from 7 studies that were performed in the Australian Twin Register (n = 3) and Netherlands Twin Register (n = 4). BP data from all of the possible quasi-independent sibling pairs with ≥1 measure in both siblings were used for linkage analysis (MZ twin pairs with an additional sibling counted once). The average time between the longitudinal measures was 12 years in Australia and 7 years in the Netherlands. Subjects were recruited on a voluntary basis from the general population. Recruitment of subjects for the first Australian study was based on word of mouth, media appeals, and advertising. The second and third studies were a follow-up of this sample, where in the third study, subjects were added who joined the Australian Twin Register during various (telephone) surveys of other studies. For the Netherlands, recruitment for the first study was through city council population registries. Subjects for the other studies were recruited through city council population registries, advertisements in twin newsletters, and other media. In consecutive Netherlands Twin Register studies, earlier participating twins might participate, and new subjects were added. The studies were approved by the appropriate ethics committees, and informed consent was obtained from all of the participants.

Measures

BP during rest was obtained using slightly different methods and included manual readings by a nurse, brachial cuff measurements...
with an automated Dynamap 845 recorder,12,14,16,17 and assessment with an ambulatory monitor (Spacelabs 90207).13 A mean of 2 to 6 BP measures was taken as the resting value. In the Dutch ambulatory BP study, the mean (3.8) of all evening measures when subjects were seated quietly was used. If subjects took antihypertensive medication, a correction of +14 mm Hg for SBP and +10 mm Hg for DBP was made for the nonambulatory studies.5,20,21 For the ambulatory study, the correction was drug class specific, and mean values were close to +14 mm Hg for SBP and +10 mm Hg for DBP.13

Genotyping

DNA was extracted from either whole blood or buccal swabs using standard protocols.22-23 Samples were genotyped by the Mammalian Genotyping Service in Marshfield, the Molecular Epidemiology Section, Leiden University Medical Centre, Sequana, or Gemini (Australian samples).24 The genotype data from these screens were combined but were kept separate for each country. Pedigree relationships were checked with Graphic Representation of Relationships.25 Errors of Mendelian inheritance were detected with Pedstats.26 Markers and samples were removed if their total error rate was >1%; in all of the other cases, the specific erroneous genotypes were set as unknown. Unlikely recombinants were detected using Merlin, and erroneous genotypes were removed with Pedwip.26 Sibling pairs were selected that had ≥200 autosomal markers genotyped for each individual. This resulted in a sample of 286 pairs for Australia. On average, 807 markers were tested per sibling (206 to 1648) with an average heterozygosity of 76% and an average centimorgan spacing of 5.6. For the Netherlands, 636 pairs were available. In the Dutch families, 544 parents were genotyped. Their information was used to obtain estimates of identity-by-descent (IBD) status in the offspring. The average number of markers genotyped in sibships was 383 (range: 201 to 743), and the average heterozygosity of autosomal markers was 76% with an average spacing of 9.7 cM. For the statistical analyses, the Haldane mapping function was used; all of the reported results were calculated using a 50:50 mixture of a \( x^2 \) distribution with a point mass of 0, and a \( x^2 \) distribution. Accordingly, a significance level of \( \alpha \) was obtained from a \( x^2 \) critical value of 2\( \alpha \).

In the joint analysis, heterogeneity and homogeneity of the QTL effect between the 2 countries was considered. Under heterogeneity, all of the model parameters \( (A, E, Q) \) between countries were freely estimated. Using a likelihood-ratio test \( (x^2) \), we first tested the assumption of homogeneity in the variance associated to a putative QTL by restricting \( Q \) to be the same across both countries while allowing other parameters to differ.30 If homogeneity of \( Q \) is observed \( (P \geq 0.05) \), a combined linkage analysis can be used next to determine the significance of the QTL effect on the basis of the pooled data set using the \( x^2 \) distribution without an increased threshold for significance (because there is no extra free parameter because of heterogeneity). Therefore, we report replicated loci with LOD scores >1.18 \( (P \approx 0.01) \), as well as new loci with an LOD >2.2 \( (P \approx 0.00074) \), the asymptotic threshold for suggestive linkage as suggested by Lander and Kruglyak.31 The 1-LOD drop support interval was used as an estimate for the 90% CI of any QTL location22 and was used to examine linkage replication compared with previously reported locations and candidate genes (using Ensembl: http://www.ensembl.org and OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM).

Results

The number of individuals, number of subjects using antihypertensive medication, male–female ratio, average age, and average BP levels are shown in Table 1. No differences in BP levels were found between MZ and DZ twins and siblings. In the later studies, an increase in age range and BP variance is seen (study 3 from Australia and studies 3 and 4 from the Netherlands). Between the 2 countries, the age range is comparable. Age and sex differences were described previously in detail.6 Briefly, SBP levels in men were ≈5 mm Hg higher than in women. DBP levels in men were generally ≈3 mm Hg higher than in women. Increasing BP with increasing age was seen in all of the studies, but the age effect did not always reach significance \( (P > 0.01) \). There were no sex differences in MZ correlations or DZ same-sex versus opposite-sex correlations \( (P > 0.01) \), suggesting that the same genes influence BP in men and women.

In Figure 1, the linkage results of the country-specific genome scans are presented for SBP. The best LOD scores of 1.67 \( (P = 0.0028) \) and 1.27 \( (P = 0.0078) \) for SBP were found at chromosome locations 5q35.1 and 11q24.2 in Australia. For the Netherlands, the highest LOD score, 1.21 \( (P = 0.0092) \), was found on chromosome 13q34 for SBP. Figure 2 shows the linkage analyses for DBP. In the Netherlands Twin Register sample, suggestive linkage was obtained for chromosome 5p13.1 with an LOD score of 2.50 \( (P = 0.00034) \). In the Australian Twin Register, this location was not replicated, however. At a lower pointwise significance level for replication \( (LOD > 1.18; P < 0.01) \), both SBP and DBP showed a signal at 5q35.1 and 11q24.2. Locations showing increased IBD sharing for either DBP or SBP were at 3p14.1, 5p13.1, 5q35.1, 7p21.3, 7q21.13, 9q33.1 to 34.2, 10p12.31, 11q13.4 to 11q24.2, 13q34, 14q12, 17p12, 17q24.3, and 19p13.3.
Table 2 summarizes the results of the joint genome scan. By combining the data of the 2 countries in a single scan, 3 locations with suggestive linkage were identified for DBP, which would not have been detected using the data in the individual countries alone. No significant heterogeneity was found for these locations, with $P$ values of 0.56, 0.93, and 0.47, respectively. The 3 loci were 5p13.1 at marker ATAG022, 14q12 at marker GATA43H01, and 17p12 at marker D17S921, with maximum LOD scores of 2.48 ($P=0.00037$), 2.40 ($P=0.00045$), and 2.36 ($P=0.00049$), respectively. For SBP, no locations with suggestive linkage were identified. Locations for the joint scan with nominal $P$s<0.01 for either SBP or DBP, which were identified previously in other studies, are also shown in Table 2. For the locations on chromosomes 5p13.1, 14q12, and 17p12, more details are depicted in Figure 3.

**Discussion**

This study presents suggestive linkage for DBP on 3 locations, namely, 5p13.1, 14q12, and 17p12. For SBP, no suggestive evidence for linkage was found. Several locations, found previously in >1 study, have been replicated to some extent.
extent (LOD: >1.18; \( P < 0.01 \)) in our study, namely, 3p14.1, 5p13.1, 5q35.1, 7p21.3, 7q21.13, 9q33.1 to 34.2, 10p12.31, 11q13.4 to 11q24.2, 13q34, 14q12, 17p12, 17q24.3, and 19p13.3.33–40 However, the significance of the loci in the individual studies is often low, the regions are broad, and the significance tends to go down when the sample size is increased.7,8 This pattern of findings can be caused by several factors, but it is likely that many genes contribute to BP variance and that each has only a small effect. With current study sample sizes, locations of these genes may be missed.31,40 To increase power, several approaches can be used, such as pooling across samples and the use of repeated measures in a longitudinal design, as was done in the present study.

Medication use may affect QTL detection, but is unlikely to have been very important here. The average age at time of participation in most studies is still relatively low for frequent use of antihypertensive medication. Roughly 4% of the subjects were using antihypertensive medication. These subjects were not excluded, because previous studies have shown that excluding medicated hypertensive persons from the analysis reduces the genetic effect and power to detect QTLs.20,21,41 Instead, we added the published treatment effects of their medication to the observed BP. When we examined which sibling pairs were responsible for the increased LOD scores at putative locations, a general finding was that concordant low BP pairs contributed most and not the pairs with high BP (data not shown). Note that this finding may be important for replication in studies examining only hypertensive pairs. Also, it indicates that there was probably a small influence of genes related to secondary forms of hypertension (caused by, eg, renal disorders or type 2 diabetes), which has not been excluded in this study.

Many candidate genes are located within the 90% CI of the 3 suggestive loci at 5p13.1, 14q12, and 17p. For the 5p13.1 location, a candidate gene is the atrial natriuretic peptide clearance receptor precursor gene (\( \text{NPR3} \)). The natriuretic peptides elicit a number of vascular, renal, and endocrine effects that are important in the maintenance of BP.42 The \( \text{NPR3} \) gene has already been associated with familial hypertension, but confirmation is still needed.43 Two other candidates in the 5p13.1 region are the insulin gene enhancer protein gene (\( \text{ISL1} \)) and the lipid phosphate phosphohydro-

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**TABLE 2. Genome-Wide Scan Locations With \( P < 0.01 \) from the Joint Linkage Analysis of the Australian and Netherlands Longitudinal Blood Pressure Studies**

<table>
<thead>
<tr>
<th>BP</th>
<th>Chromosome</th>
<th>Position, cM</th>
<th>Marker</th>
<th>No Heterogeneity</th>
<th>Homogeneity Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( P )</td>
<td>LOD ( P )</td>
</tr>
<tr>
<td>DBP</td>
<td>3p14.1</td>
<td>88.2</td>
<td>D3S1285</td>
<td>0.40</td>
<td>1.60</td>
</tr>
<tr>
<td>DBP</td>
<td>5p13.1</td>
<td>60.8</td>
<td>ATAG022</td>
<td>0.56</td>
<td>2.48</td>
</tr>
<tr>
<td>DBP</td>
<td>9q33.1</td>
<td>121.6</td>
<td>D9S1776</td>
<td>0.36</td>
<td>1.54</td>
</tr>
<tr>
<td>DBP</td>
<td>9q34.2</td>
<td>154.9</td>
<td>CTAT016</td>
<td>0.20</td>
<td>1.59</td>
</tr>
<tr>
<td>DBP</td>
<td>11q13.4</td>
<td>84.3</td>
<td>D11S2371</td>
<td>0.41</td>
<td>1.73</td>
</tr>
<tr>
<td>SBP</td>
<td>13q34</td>
<td>113.7</td>
<td>D13S1315</td>
<td>0.67</td>
<td>1.21</td>
</tr>
<tr>
<td>DBP</td>
<td>14q12</td>
<td>19.6</td>
<td>GATA49H01</td>
<td>0.93</td>
<td>2.40</td>
</tr>
<tr>
<td>DBP</td>
<td>17p12</td>
<td>43.1</td>
<td>D17S921</td>
<td>0.47</td>
<td>2.36</td>
</tr>
<tr>
<td>DBP</td>
<td>17q24.3</td>
<td>109.8</td>
<td>GATA63G01</td>
<td>0.66</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Heterogeneity testing: \( P \) value for the test of the assumption that there is no heterogeneity of the QTL effect between the 2 countries. Linkage under the homogeneity model: QTL analysis under the assumption that an equal amount of variance is explained by the QTL in the 2 countries. Positions in Kosambi centimorgans.27
lase 1 gene (PPAP2A). Insulin metabolism, lipid metabolism, and BP regulation are strongly intertwined, and detrimental shifts in these risk factors for cardiovascular disorder often co-occur (the metabolic syndrome), possibly because of a joint genetic underpinning.44–46

For the second region on chromosome 14q12, 2 candidate genes were found, namely the low-density lipoprotein receptor–related protein 10 precursor gene (LRP10) and the chymase precursor gene (CMA1). The second is related to the angiotensin pathway and causes hypertension and arteriopathy in rats when the gene is upregulated.47 However, in a large study with Japanese subjects, variations in this gene did not show any association with BP.48

For the third region, 17p12, the 90% CI is substantial. Several studies have indicated that there is probably >1 gene regulating BP on chromosome 17.49 Because BP depends on salt balance, allelic variants of genes that influence this balance can be considered as potential candidates. In the 17p12 region, there are several of those genes, namely the NO synthase IIB and IIC genes (NOS2A and NOS2B), the solute carrier family 13-member 2 renal sodium/dicarboxylate cotransporter gene (SLC13A12), the solute carrier family 5 sodium/glucose cotransporter member 10 gene (SLC5A10), and the ATP-sensitive inward rectifier potassium channel 12 gene (KCNJ12).49–51 Other candidates are genes involved in syndromes caused by more severe DNA alterations; 2 located near 17p12 are neurofibromatosis (NF1 gene) causing renal vascular damage52,53 and platelet glycoprotein IV deficiency (CD36 gene) leading to a changes in all of the factors involved in the metabolic syndrome.54,55 Another candidate involved in lipid metabolism within the 17p12 region is the fatty aldehyde dehydrogenase gene (ALDH3A2). Furthermore, at each candidate location, genes related to the ubiquitin–protein ligase family were present. Ubiquitin has been linked to angiotensin II pathways and Na+/H+ channel function in endothelial cells.56–58 These genes could, therefore, be considered potential candidates as well.

**Perspectives**

In this study, we found suggestive linkage for DBP at 5p13.1, 14q12, and 17p12. These loci have been replicated, and our study indicates further evidence for their involvement. Fine mapping of these regions, preferably by dense coverage of single nucleotide polymorphism markers, followed by family based association studies, is now a first priority. Next, functional variants that predispose to hypertension or a lower BP instead can be identified, as well as the pathways involved.

**Figure 3.** Detailed maps of the chromosomes with suggestive linkage for DBP. On the horizontal axis, the position on the chromosome is indicated in Kosambi centimorgans. On the vertical axis, the LOD score is given. The vertical dotted lines indicate the 90% CI around the maximum LOD score. On top, the tested markers are presented and in the right corner the chromosome band.
Acknowledgments

We thank Nina Kupper, Mireille van den Berg, Harold Snieder, and Eline P. Slagboom for their contributions to phenotyping and genotyping.

Sources of Funding

We would like to acknowledge the following grants: Genetics of Cardiovascular Disease and Stress-Physiology of Coronary Heart Disease (Netherlands Heart Foundation grants 86.083 and 88.042 90.313; Human Frontiers of Science Program RGO154/1998-B; Genetic Basis of Anxiety and Depression (Netherlands Organisation for Scientific Research [NWO] 904-01-090; Spinozapremie (NWO SPI 56-464-14192); Twin-Family Database for Behavior Genetics and Genomics (NWO 480-04-004); Centre Neurogenetics and Cognition Research; Center Medical Systems Biology (NWO Genomics); Genome-Wide Analyses of European Twin and Population Cohorts (EU/QLRT-2001-01254); Genome Scan for Neuroticism (National Institute of Mental Health grants R01 MH059160); National Institutes of Health grants (DA00272, DA12854, DA12540, CA75581, AA07535, and AA07728); and Australian National Health and Medical Research Council grants (971123 and 941177). D.P. is supported by NWO/VIDI (452-05-318).

Disclosures

None.

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 Genome-Wide Scan for Blood Pressure in Australian and Dutch Subjects Suggests 
 Linkage at 5P, 14Q, and 17P
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Hypertension. 2007;49:832-838; originally published online February 26, 2007; 
doi: 10.1161/01.HYP.0000260092.93964.ed

The online version of this article, along with updated information and services, is located on the 
World Wide Web at: 
http://hyper.ahajournals.org/content/49/4/832

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