Genome-Wide Scan for Blood Pressure Suggests Linkage to Chromosome 11, and Replication of Loci on 16, 17, and 22

Marlies de Lange, Tim D. Spector, Toby Andrew

Abstract—Hypertension was one of the first complex traits to be studied and is thought to be influenced by polygenic and multiple environmental risk factors. Several genomic studies have found suggestive logarithm of odds (LOD) scores for either blood pressure or essential hypertension, but few loci have been replicated. In this study, we performed a genome-wide linkage analysis for systolic blood pressure (SBP) and diastolic blood pressure (DBP) on 1109 white female dizygotic twin pairs from the TwinsUK registry in London. Multipoint linkage analysis replicated the locations of 3 previously reported linkage peaks: on chromosome 16 at 65 cM (LOD 0.8 for SBP and 1.8 for DBP); on chromosome 17 at 70 cM (LOD 1.8 SBP); and at 35 cM on chromosome 22 (LOD 0.97 SBP and 0.99 DBP). Results from multipoint analysis showed 1 novel suggestive linkage for SBP (multipoint LOD 2.28; 2-point P=0.0007) at 35 cM on chromosome 11. Results were similar when those on blood pressure medication were excluded. These are encouraging results for hypertensive research and demonstrate that despite past disappointments, linkage studies can be used to replicate regions from other studies and potentially discover new genetic risk factors of moderate to large effect size. Considering the differences in selection and ascertainment of the previous linkage studies, these results also suggest that some quantitative trait loci are likely to influence the normal range of blood pressure and clinical hypertension, whereas others will be specific to each trait. Future studies should focus on the fine mapping of these replicated regions, which include potential candidate genes. (Hypertension. 2004;44:872-877.)

Key Words: genetics ■ linkage analysis ■ blood pressure ■ twins

Researchers are still not close to understanding the genetic influences on complex diseases such as hypertension. The small individual genetic effects expected to act on complex disease are not only difficult to find,1 but demonstrating a functional role for potential candidate genes is harder still. Although blood pressure is known to be a heritable trait, the modest reported heritability of ≈50% for blood pressure and hypertension (53% for systolic blood pressure [SBP] and 48% for diastolic blood pressure [DBP] in our sample2), coupled with the number of genetic studies performed to date, does perhaps make finding quantitative trait loci (QTLs) of large effect less likely.

Several genome-wide scans on hypertension3–5 and on healthy variation in blood pressure6–10 have been performed recently. For the latter design, genetic informativeness is enhanced by selection, for example, by using extremely discordant or concordant sib-pairs for blood pressure,11,12 or by sampling families of probands with young-age elevated blood pressure.13 However, reported logarithm of odds (LOD) scores generally do not exceed the recommended threshold for genome-wide statistical significance,14 which makes it all the more important that replication studies confirm any suggestive linkages found in comparable studies.
perhaps unfeasible family studies to reliably detect small genetic effects, the best option is to use currently available studies to replicate suggestive linkage results, ideally by combining the raw data from different studies or by using the same named genetic markers.

In this study, we performed a genome-wide linkage analysis for SBP and DBP on >1000 white female pairs of twins, including 213 women on antihypertensive medication.

**Methods**

**Subjects**
A total sample of 2818 dizygotic (DZ) female twins (1394 complete twin pairs) aged 18 to 79 years who visited the Twin Research Unit between June 1992 and October 1999 had at least 1 reliable measurement of SBP and DBP taken at rest in sitting position 10 minutes after an initial discarded test reading using the automated Omron machine. Although some twins had multiple measurements at several time points, many twins had only 1 available valid blood pressure (BP) measure. Therefore, the first measure of all twins was used for consistency in analysis. A total of 1109 complete twin pairs with valid BP information also had genotypic information from a genome-wide marker scan.

Twins from the registry were ascertained from the general population through national media campaigns in the United Kingdom for a wide variety of studies of common diseases and traits. Participating twins were unaware of the specific hypotheses tested, and informed consent was obtained from all subjects. The study was approved by the St. Thomas` Hospital research ethics committee. Initial zygosity was determined by standardized questionnaire, and DNA fingerprinting was used for confirmation. Information on medication use and demographic variables was obtained by standardized nurse-administered questionnaire. A subgroup of 9.7% twins were on medication influencing their BP such as β-blockers and antihypertensive medication. BP measures for these twins were incremented by 10 mm Hg for SBP and 5 mm Hg for DBP for the expected therapeutic effect of drug treatment, as observed in previous studies, to allow the inclusion of these informative subjects in the analysis.

**Genotyping**
DNA was extracted at the Twin Research Unit Laboratory using the BACC2 DNA extraction kit (Nucleon Biosciences) from 10 mL of venous blood collected on 1.6 mg/mL EDTA. Genome-wide linkage analysis included 737 genotypic markers equivalent to highly polymorphic microsatellite markers spaced every 10 cM using standard fluorescence-based genotyping methodologies from the ABI Prism linkage mapping set (Applied Biosystems) and Genethon Genetic Linkage Map and described in more detail previously. The estimated genotyping error rate was <1%.

**Statistical Analysis**
Genome-wide linkage analysis was conducted using generalized linear modeling in STATA. This regression technique is based on optimal Haseman and Elston methods, in which the square of the sibling difference in SBP and DBP (adjusted for age) is regressed on the estimated proportion of alleles identical-by-descent (IBD). Age-adjusted residuals were used in the analysis to address heteroscedasticity in BP because the variance of BP increased with age (data not shown). The estimated proportions of alleles shared IBD by a twin pair was obtained using Genehunter 2 software.

First, multipoint linkage analysis was performed on the total DZ twin sample, including individuals on antihypertensive treatment. Genome-wide significance thresholds were used for the genome scans. Nominal significance thresholds (P < 0.05) were used to replicate previous linkage peaks, but the replicate location was defined strictly to be within ± 3 cM using the Genethon genetic map. To check for the influence of individuals on hypertensive medication, multipoint linkage analysis was also performed on the untreated sample, in which individuals on BP-influencing medication were removed from the analysis. Results from the untreated twin sample were very similar and are not reported unless the untreated sample was more relevant for comparison with the protocol of previously reported linkage studies.

Second, 2-point microsatellite linkage analyses were used to confirm multipoint linkage results (ie, for the 1 novel suggestive peak observed in this study), as well as examining regions of previously reported linkage (Table 1, summary). Results from 2-point linkage were checked for model fit using standard regression diagnostics, and linkage results were confirmed with outliers removed.

Two genome-wide scans were performed: 1 for SBP and 1 for DBP. However, because SBP and DBP are highly correlated (r = 0.74), the effective number of independent “experiments” was estimated to be 1.45 rather than 2. Hence, significance thresholds were not revised downward for this study.

**Results**
Table 2 presents the general characteristics of the DZ twins with BP measurements. There was no significant difference in either SBP or DBP for the larger total of DZ twins measured for BP compared with the subset of twins with valid BP and genomic data (data not shown), suggesting no phenotypic selection bias for our genome scan. As expected, mean age of the total sample, including the twins on antihypertensive treatment, was higher compared with untreated twins. The means for SBP and DBP in the total sample including twins on antihypertensive treatment was slightly higher than the untreated subgroup, with a larger corresponding increase in variance in BP for the total group, including subjects on antihypertensive treatment.

**TABLE 1. Summary of Linkage Literature on BP Replicated in This Study**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Measurement</th>
<th>Sample</th>
<th>Chromosome</th>
<th>Marker</th>
<th>cM (Marshfield)</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu et al</td>
<td>1999</td>
<td>Low SBP</td>
<td>Extremely concordant and discordant Chinese sib-pairs</td>
<td>16</td>
<td>D16S3396</td>
<td>63.78</td>
<td>2.74</td>
</tr>
<tr>
<td>Atwood et al</td>
<td>2001</td>
<td>SBP and DBP</td>
<td>Mexican-Americans</td>
<td>16</td>
<td>D16S3253</td>
<td>71.77</td>
<td>1.21; 1.84</td>
</tr>
<tr>
<td>Levy et al</td>
<td>2000</td>
<td>SBP and DBP</td>
<td>Framingham Heart Study</td>
<td>17</td>
<td>D17S1299</td>
<td>62.01</td>
<td>3.8; 1.6</td>
</tr>
<tr>
<td>Levy et al</td>
<td>2000</td>
<td>SBP</td>
<td>Framingham Heart Study</td>
<td>17</td>
<td>D17S2180</td>
<td>66.85</td>
<td>3.1</td>
</tr>
<tr>
<td>Perola et al</td>
<td>2000</td>
<td>Affection status</td>
<td>Finnish affected sib-pairs</td>
<td>22</td>
<td>D22S685</td>
<td>32.4</td>
<td>MLS 2.07</td>
</tr>
<tr>
<td>Krushkal et al</td>
<td>1999</td>
<td>SBP</td>
<td>Discordant Sib-pairs</td>
<td>2</td>
<td>D2S1788</td>
<td>55.51</td>
<td>P = 0.0089</td>
</tr>
<tr>
<td>Caulfield et al</td>
<td>2003</td>
<td>Hypertension</td>
<td>British affected siblings from severely hypertensive families</td>
<td>9</td>
<td>D9S290</td>
<td>140.86</td>
<td>2.24</td>
</tr>
</tbody>
</table>

MLS indicates maximum likelihood score.
Results From Multipoint and 2-Point Linkage Analysis

Figure 1 presents LOD scores for SBP and DBP from genome-wide multipoint linkage analysis on the total DZ twin sample. Only 1 marker on chromosome 11 at 35 cM reached a suggestive genome-wide LOD score threshold at LOD 2.28 for SBP (Table 3). At this locus, 2-point analysis for SBP showed highest significance for marker D11S904 (P_H11005 0.0007; LOD 2.5). Although there was nominally significant linkage to D11S904 for DBP (P=0.014), this result appeared to have been influenced by a few outliers, and subsequent removal of 3 twin pairs resulted in a reduced P value of 0.06. Potential novel linkage results falling just below the suggestive threshold can also be seen on chromosomes 6, 7, and 12 in Figure 1.

Table 1 presents a summary of BP and hypertension genome-wide scans published in the last few years, with replicated peaks from this study shown in Table 3. A replication of the 65-cM region on chromosome 16 was seen for SBP and DBP, with respective LOD scores of 0.8 and 1.82 (both nominally significant at P=0.05 and P=0.004, respectively). Two-point analysis confirmed linkage to marker D16S415 (P=0.006) for DBP and SBP (P=0.015). Replication of the 70-cM region of chromosome 17 was seen for SBP with LOD scores of 1.8, but only LOD 0.67 for DBP and 2-point linkage failed to reach significance for the microsatellites in that area. Maximum LOD scores of 0.97 for SBP and 0.99 for DBP were seen for a reported linkage to chromosome 22 in the 35-cM region. Two-point linkage was of borderline significance for SBP (P=0.05) and not very stable for DBP, in which the 2-point P value for marker D22S283 increased from 0.012 to 0.085 when 3 outliers were removed.
Two further possible peaks that are in a similar region but may not correspond exactly with previous reports deserve a closer look in new linkage scans. These include the 145- to 150-cM region on chromosome 9 (from the BRIGHT study), which, for this study, shows an LOD of 1.5 in the total sample and an LOD of 1.07 excluding treated individuals. Similarly, an LOD of 1.3 for SBP was observed at 50 cM on chromosome 2 in this study in a region 5.5 cM away from Krushkal et al. Generally, the linkage results (multipoint and 2-point) were very similar when only the untreated subgroup of twins was analyzed (data not shown).

Discussion

This study presents 1 new area of suggestive linkage at 35 cM on chromosome 11 and replicates 3 areas of previous suggestive linkage. These are encouraging results for hypertension research and demonstrate that with care, current linkage studies can be used to replicate loci from other studies and may in future uncover new genetic risk factors of moderate to large effect size. Ideally, to maximize statistical power, linkage results need to be confirmed by pooling data from different studies. It has been argued that genes for BP may have individual small effect sizes and therefore will be missed in most studies of average size using the stringent LOD scores (>3.2) recommended for evidence of linkage and an LOD of >2.2 for suggestive linkage in genome-wide studies. We agree with this assessment. The threshold for significant replication can be set lower (an LOD score of >0.8, equivalent to P<0.05), where there is good previous evidence for a candidate marker, because stringent rules correcting for multiple testing do not apply, provided the location is identified using the same named marker, or failing this, located using genetic distance (for this study, ≤3 cM).

To illustrate this point, Figure 2a presents the (lack of) power to detect small QTL effects at genome-wide significance level (P=0.0001) for different samples (derived using a Genetic Power Calculator; www.iop.kcl.ac.uk/gpc). From this figure, it is clear that only large sample sizes of, for example, 2500 families, each with 4 siblings, are adequately equipped to detect small QTL effects of <10%. However, replication is by far the best way to rule out false-positives as well as to estimate the true effect size in a less biased way. The power to detect a QTL at a nominal significance level (P=0.05) improves considerably (Figure 2b) if justified by previous evidence because the penalty of multiple testing is greatly reduced. Nevertheless, the figure also indicates our sample is still only sufficiently powered to replicate QTLs of large effect: a sample of 1100 sibships has 80% power to detect QTL effects of ≥15% (Figure 2b). However, it is possible that linkage studies could pick up signals

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Power to detect novel QTL effects (a, $\alpha=0.0001$; equivalent to LOD 3.2) and replicate previously observed QTL linkage peaks (b, $\alpha=0.05$; equivalent to LOD 0.8). Both graphs assume complete marker informativeness and a recombination fraction of zero between the marker and the QTL.

### Table 3. Multipoint and 2-Point Genome-Wide Linkage Results for SBP and DBP

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>cM</th>
<th>LOD SBP</th>
<th>LOD DBP</th>
<th>N</th>
<th>Marker</th>
<th>P Value SBP</th>
<th>P Value DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>New region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>35</td>
<td>2.28</td>
<td>0.90</td>
<td>1028</td>
<td>D11S904</td>
<td>0.0007</td>
<td>0.014*</td>
</tr>
<tr>
<td>Replication regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>65</td>
<td>0.80</td>
<td>1.82</td>
<td>1030</td>
<td>D16S415</td>
<td>0.015</td>
<td>0.006</td>
</tr>
<tr>
<td>22</td>
<td>35</td>
<td>0.97</td>
<td>0.99</td>
<td>1028</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>70</td>
<td>1.8</td>
<td>0.67</td>
<td>1030</td>
<td>D22S283</td>
<td>0.05</td>
<td>0.012*</td>
</tr>
</tbody>
</table>

N indicates No. of complete pairs.

*Dependent on 2 or 3 influential twin pairs.
of this order of magnitude from a number of genes of small effect clustered together at a locus.

A replication at the 65-cM region on chromosome 16 was seen for SBP and DBP. The QTL(s) showed evidence of 2-point linkage to marker D16S415 for DBP (Table 3), which is located between the markers D16S396 and D16S3253 from previous studies (Table 1). Marker D16S3396 has been reported for linkage in a study of low-concordant SBP Chinese siblings\(^8\) and linkage to marker D16S3253 for SBP and DBP in unselected Mexican-American families.\(^7\) Moreover, multipoint linkage analysis using only untreated subjects produced consistent LOD scores in this area with maximum LOD scores of 1.39 for SBP and 2.39 for DBP (lower LOD scores were obtained for the total group). This is consistent with the initial significant reports of the 65-cM area of chromosome 16, which were ascertained from the normal population,\(^7,12\) and not via probands of hypertensive families. In contrast, the original linkage to the 35-cM region on chromosome 22 for high DBP was obtained through concordant affected Finnish siblings with relatively young age (<50) at diagnosis of high DBP.\(^3\) The distance between their marker (D22S685) and ours (D22S283) is ~2 Mb. In our data, the LOD score for the total and untreated samples showed little or no difference for DBP. It is likely that hypertensive and untreated individuals could show genetic heterogeneity for some loci, and this might be 1 reason that we do not seem to replicate the exact loci from the highly selected hypertensive families in the BRIGHT study.\(^4\) One encouraging finding is that the loci we do replicate appear to be observed in several populations (ie, Mexican-Americans, Chinese, and whites).

The replication result of SBP to the 70-cM region on chromosome 17 is a little less clear cut. Levy et al\(^8\) reported a multipoint LOD of 4.7 to the 67-cM region on chromosome 17 for SBP, with a 2-point LOD of 3.1 for marker D17S2180. Although we were unable to replicate the 2-point linkage results using nearby neighboring markers, we do observe a multipoint LOD score of 1.8 for SBP in this region. To our knowledge, no other linkage studies have highlighted this region. It would be interesting to see the multipoint and 2-point results of other genomic linkage studies, preferably including the data of individuals on antihypertensive treatment as ours and Levy et al\(^8\) have. It could be that this locus influences the BP of healthy and hypertensive individuals, but linkage might be stronger for hypertensive families. Under these circumstances, removing potentially informative hypertensive sib-pairs would result in a reduction of power to detect loci.\(^20,30\) Considering the wide differences in ascertainment of linkage studies conducted so far, these results suggest that there appear to be loci to influence the normal range of BP as well as those leading to hypertension.

This study was able to replicate previous reported areas, although the BP measurement protocol we used for the genome analysis did not include multiple BP measures during 1 visit, as did all of the reported linkage studies, but only 1 measure. Despite using a combined set of ABI and Genethon genotypic markers, for most comparisons with previous 2-point linkage analyses, it was not possible to use exactly the same marker but the nearest neighboring microsatellite. In addition, the information content derived from some markers in our map, although >70% for most of the genome, will be below this for some areas.

However, our study has some advantages. The number of siblings in this study is larger than other sibling genome-wide scans, and being twins, they are matched for age and closely matched for other environmental influences and cultural factors during childhood such as diet, salt intake, and exercise patterns. Age is an important risk factor for BP, and matching within families helps reduce confounding. In addition, because twins are matched for age, DZ twins are likely to have more shared environmental effects in common than ordinary siblings, resulting in a larger between-family component of variance, so that the proportion of within-family variance that is explained by the QTL, and hence power, may be larger for twin pairs.\(^32\)

Because we have replicated loci reported previously using highly selected samples for hypertension, this study suggests the idea that the results for some loci might be applicable to healthy and affected samples. However, 1 or more large comprehensive linkage studies incorporating severely hypertensive as well as healthy families would be necessary to check whether the same markers in these regions of interest are clearly linked in both types of samples. Based on BP levels and lifestyle factors such as prevalence of smoking, we have demonstrated previously\(^33\) that our twins are generalizable to singletons for common cardiovascular disease traits. Any effects of twinship, for example, caused by in utero factors or birth weight, are minimal\(^18\) and are unlikely to affect linkage results.

Future studies should focus on the fine mapping of these replicated regions for candidate genes. Although each area contains many potential candidates, a few immediately stand out. Atwood et al\(^7\) reported the occurrence of the thiazide-sensitive Na-Cl cotransporter gene in the 65-cM region on chromosome 16. Further linkage disequilibrium studies using an understanding of the full variation in this gene is necessary to assess its potential influence on BP. At 22q13.1 is the apolipoprotein A-1 gene involved in the formation of most cholesterol esters in plasma as well as promoting efflux of cholesterol from cells. Because elevated BP is 1 symptom of the insulin resistance syndrome, 1 explanation could be that some genes have pleiotropic action on lipid levels and BP.\(^34\) Another gene in the same region of 22q13.1 is a member of the neuronal calcium channel γ-subunit gene subfamily of the PMP-22/EMP/MP20 family and is thought to stabilize the calcium channel in an inactive (closed) state. In the area of the markers on chromosome 17 reported by Levy et al,\(^8\) a peroxisome proliferator-activated receptor-binding protein is located, which could be a regulatory candidate. Although the suggestive linkage detected at 35 cM on chromosome 11 needs to be replicated, some candidates make this region potentially interesting. Closely, an Na+/solute symporter is located as well as BBOX1, which catalyzes the formation of L-carnitine from γ-butyrobetaine, which is essential for transport of activated fatty acids across the mitochondrial membrane during mitochondrial β-oxidation and is highly expressed in the kidney.

In conclusion, this study shows that linkage results are possible to replicate and lends encouragement for future linkage studies for other complex traits. Association studies using fine maps of replicated regions are now of major priority to achieve the realistic goal of identifying genes influencing variation in BP.
Perspectives

The replication of linkage results for complex traits is difficult, and the reasons for this are well documented.\textsuperscript{1,6} Linkage replication requires strict criteria, preferably by combining data from different studies rather than relying on summary statistics, but where this is not possible, a minimal criterion is use of the same-named markers. In this study, our results provide further evidence of linkage to 3 genomic regions, in particular 16q12.2, harboring loci-influencing SBP and DBP. Fine mapping of replicated regions are now a major priority to achieve the goal of identifying functional variants that predispose individuals to hypertension and coronary heart disease.

Acknowledgments

The Twin Research and Genetic Epidemiology Unit is supported by grants from the Wellcome Trust, Chronic Disease Research Foundation, Arthritis Research Campaign, and European Union (EU) framework. We thank the research nurses for skillful data collection and the twins for their continued support. This study is conducted as part of the GenomEUtwin project (QLG2-CT-2002-01254). We would also like to thank 2 anonymous reviewers for their helpful comments.

References

Genome-Wide Scan for Blood Pressure Suggests Linkage to Chromosome 11, and Replication of Loci on 16, 17, and 22

Marlies de Lange, Tim D. Spector and Toby Andrew

Hypertension. 2004;44:872-877; originally published online November 8, 2004;
doi: 10.1161/01.HYP.0000148994.89903.fa

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/44/6/872

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/