Genome-Wide Linkage Analysis of Pulse Pressure in Mexican Americans

Larry D. Atwood, Paul B. Samollow, James E. Hixson, Michael P. Stern, Jean W. MacCluer

Abstract—Pulse pressure, a measure of aortic stiffness, is a strong predictor of cardiovascular mortality. To locate genes that affect pulse pressure, we performed genetic analysis on randomly ascertained families in the San Antonio Family Heart Study. Pulse pressure was defined as the difference between systolic and diastolic blood pressures. Likelihood methods were used to construct a model that had both single-locus and polygenic components for 46 families (1308 individuals). The single-locus component included sex-specific and genotype-specific effects of both age and body mass index. Using this model, we then performed 2-point linkage analysis in 10 families (440 individuals) that were among the largest of the 46 families and that had been genotyped for 399 polymorphic markers. The model that contained only the polygenic component and simple effects of the covariates showed pulse pressure heritability of 0.21. When the single-locus component was added, the sex-specific and genotype-specific effects of age and body mass index were highly significant ($P<0.002$). The full model accounted for 73% of the total variation of pulse pressure. Linkage analysis using this model with each marker revealed 4 markers with lod scores $>1.9$, which is the Lander-Kruglyak suggestive linkage standard. D21S1440 had a lod score of 2.78 with a recombination fraction ($\theta$) of 0.02. D7S1799 had a lod score of 2.04 ($\theta=0.01$), D8S1100 had a lod score of 1.98 ($\theta=0.08$), and D18S844 had a lod score of 1.95 ($\theta=0.11$). These results are highly correlated with results involving systolic blood pressure, indicating that pulse pressure may not be genetically distinct from systolic blood pressure. (Hypertension. 2001;37[part 2]:425-428.)

Key Words: population ■ genetics ■ blood pressure ■ hypertension, genetic ■ cardiovascular diseases

Pulse pressure (PP), a measure of aortic stiffness, is a strong predictor of cardiovascular morbidity and mortality. An association between PP and cardiovascular events has been shown in both normotensive and hypertensive subjects. Among individuals aged $>65$ years, PP is the best measure of blood pressure that predicts mortality. Unfortunately, little is known about the genetics of PP.

The strong effects of sex, age, and body mass index (BMI) on blood pressure in the general population are well known. The high correlation between systolic blood pressure (SBP) and PP suggests that these effects exist for PP as well. In most genetic analyses, a simple correction that is held constant across genotypes is used to account for these effects. A possible interaction between genes and PP has not been reported in any study. Indeed, only a few studies have looked for interactions between common covariates and SBP or diastolic blood pressure (DBP). A segregation analysis of a large number of randomly ascertained nuclear families showed that there were significant genotype-specific effects of both sex and age on SBP. Recently, Turner et al have shown that the ACE insertion/deletion polymorphism has genotype-specific effects on blood pressure. Furthermore, Kardia has called for a more explicit consideration of gene-gene and gene-environment interactions in the study of blood pressure traits. In the present study, we modeled PP with sex-specific and genotype-specific effects of age, age$^2$, and BMI. Then, using the resulting model, we performed genome-wide 2-point lod score ($\zeta$) analysis on PP. We used conservative criteria for claiming significant ($\zeta>3.3$) and suggestive ($\zeta>1.9$) linkage. We also report results that are weakly suggestive ($\zeta>0.59$, which corresponds to a value of $P<0.05$). Given the known high correlation between PP and SBP, we also compare the genome scans for the 2 measures.

Methods

Subjects
Probands in the San Antonio Family Heart Study (SAFHS) were 40- to 60-year-old men and women chosen at random from a low-income Mexican American barrio in San Antonio, Tex. Apart from age, there were 2 eligibility criteria: (1) the proband had a living spouse who was willing to participate in the study, and (2) the proband had at least 6 first-degree relatives, excluding parents, who were aged $\geq16$ years and living in the San Antonio area. Because these criteria show no obvious selection bias with respect to blood pressure, this data set can be considered a random sample from the San Antonio Mexican American population. The proband and all first-, second-, and third-degree relatives were recruited.

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third-degree relatives willing to participate were subjected to an extensive data-gathering protocol in which demographic information on morphometrics, cigarette smoking, alcohol consumption, dietary behavior, and physical activity were obtained. For all individuals, blood pressure was measured 3 times after a 5-minute seated rest period with a random zero sphygmomanometer. Blood pressure was calculated by dropping the first reading and averaging the latter 2 readings. PP was calculated as the difference between SBP and DBP. The model-building phase of the study included 41 extended families. The total number of individuals was 1777 (1308 with blood pressure data). Several families had marriages with no children with complete data. Removing these marriage links occasionally had the effect of turning 1 large family into multiple smaller families that are computationally more efficient. This increased the actual number of families analyzed to 46.

The SAFHS chose 10 families for initial genotyping, and the linkage reported in the present study is based on these 10 families. These 10 families (637 individuals, 495 with PP data) were chosen, on the basis of size, to maximize statistical power to detect linkage. Inasmuch as this selection shows no obvious bias with respect to PP, we regard the 10 families as a random sample with respect to PP. However, it must be recognized that this selection procedure may have introduced hidden bias with respect to PP.

All individuals who participated in SAFHS gave informed consent, and the Institutional Review Board of the University of Texas Health Science Center at San Antonio approved all protocols.

Genotyping
Genotyping of 399 polymorphic markers was accomplished by using standard methods described by Atwood et al. Polymorphic markers used were from the MapPairs 6 and 8 Linkage Screening Sets (Research Genetics, Inc). An AGT 3′ dinucleotide polymorphism was typed as described by Atwood et al.11 There were 399 polymorphic markers, both candidate loci and anonymous marker loci, that had heterozygosity of ≥0.60 and were included in the linkage analysis. The average number of genotyped individuals per marker used in the linkage analysis was 441 (SD 19) in 10 families. To save computation time in the linkage analysis, any marker that had ≥7 alleles was reduced to 7 alleles by using the DOWNCODE program, which minimizes information loss. The average heterozygosity of all included dialleled markers was 0.75 (SD 0.06), ranging from 0.60 to 0.85. All genetic locations used in the present study are given in centimorgans from the p-terminus and are taken from the Genetic Location Database.13 The Genetic Location Database is the only database that contained all markers of interest and therefore provides consistent location estimates.

Statistical Analysis
Using version 4 of the Pedigree Analysis Package,14 we then performed 2-point linkage analysis on the 10 SAFHS families that had been genotyped. In the linkage analysis, one locus that was constructed on all 46 families was described by the mendelian model, and the other locus was 1 of the 399 polymorphic markers. Marker allele frequencies were estimated from the data. Parameter estimates for both loci were fixed; thus, the only free parameter in the linkage model was the recombination fraction (θ). The lod score (z) at the maximum likelihood estimate of θ was computed as logL(θmax)−logL(θ=0.5). For any significant linkage, we also tested for sex-specific recombination and heterogeneity between families.

Results
Tests of model parameters for PP showed that age and age² were significant as both sex-specific and genotype-specific effects (P=1.65×10⁻⁵ and P=1.79×10⁻⁵, respectively). BMI also had significant sex-specific and genotype-specific effects (P=1.96×10⁻³ and P=1.94×10⁻³, respectively). The full model was therefore the most parsimonious model and was used in the linkage analysis.

The Table shows the maximum lod score and recombination fraction for all markers with maximum lod scores >0.59 (equivalent to P=0.05 for a 1-tailed test) for PP. There were no markers with a lod score >3.3, which is the criterion for significant linkage in families proposed by Lander and Kruglyak.8 However, there were 4 markers with maximum lod scores >1.9, which is their criterion for suggestive linkage in families. D21S1440 had a maximum lod score of 2.78 at a θ value of 0.02, D7S1799 had a maximum lod score of 2.04 (θ=0.01), D8S1100 had a maximum lod score of 1.98 (θ=0.08), and D18S844 had a maximum lod score of 1.95 (θ=0.11). The lod scores for flanking markers were generally low, with the highest coming on the p-terminus of D8S1100 at marker D8S373, which had a maximum lod score of 0.59.

The raw variance of PP was 234.9. The polygenic model, ie, the model with no single locus component, yielded a heritability of 0.21 for PP and a residual variance of 128.4

found, the model was maximized 500 times with different random initial estimates.

Is the maximum likelihood model valid for linkage analysis? Blood pressure is a complex trait, and the single locus model is certainly incorrect. However, Williamson and Amos16 have shown that linkage analysis is valid when the genetic trait model is incorrect but the marker model is correct. Because this sample shows no selection bias with respect to blood pressure, allele frequencies based on the data are a random sample of the population; ie, the marker model is correct. Therefore, the linkage analysis is valid. Would a major gene from a prior segregation analysis improve power? An analysis of Genetic Analysis Workshop 9 data11 showed that requiring a major gene from a segregation analysis before linkage analysis can actually reduce overall power to detect linkage for a complex trait. Thus, a segregation analysis is not necessary to achieve a valid error rate and may reduce power; therefore, we did not perform one on this data set.

To determine whether the sex-specific and genotype-specific parameters were significant, several subset models were analyzed to test these parameters with respect to age, age², and BMI. In these subsets, the sex-specific or genotype-specific parameters were removed, and the maximum likelihood of the resulting subset model was computed. Examination of all possible subsets is computationally impractical, so we tested subsets grouped by age (this group included both age and age²) and BMI, in that order. All subsets were compared with the full 27-parameter model by a χ² test. If a sex-specific or genotype-specific component was found to be non-significant, it was removed from further consideration.

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Markers with Lod Scores ≥0.59 (P=0.05) for PP

<table>
<thead>
<tr>
<th>Marker</th>
<th>Recombination</th>
<th>Lod Score</th>
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</thead>
<tbody>
<tr>
<td>D1S552</td>
<td>0.09</td>
<td>0.83</td>
</tr>
<tr>
<td>D1S1595</td>
<td>0.15</td>
<td>0.81</td>
</tr>
<tr>
<td>D2S1780</td>
<td>0.15</td>
<td>0.66</td>
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<tr>
<td>D2S1790</td>
<td>0.16</td>
<td>1.28</td>
</tr>
<tr>
<td>D2S442</td>
<td>0.14</td>
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</tr>
<tr>
<td>D2S1399</td>
<td>0.22</td>
<td>0.63</td>
</tr>
<tr>
<td>D2S1363</td>
<td>0.23</td>
<td>0.68</td>
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<tr>
<td>D2S2297</td>
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<td>0.96</td>
</tr>
<tr>
<td>D2S427</td>
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<td>D2S125</td>
<td>0.18</td>
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<tr>
<td>D3S1285</td>
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<td>D5S629</td>
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<td>D7S1799</td>
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<td>D8S1110</td>
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<td>D8S1179</td>
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<td>0.59</td>
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<tr>
<td>D8S1100</td>
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<tr>
<td>D8S373</td>
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<tr>
<td>D11S4464</td>
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<tr>
<td>D12S392</td>
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<td>0.84</td>
</tr>
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<td>D14S611</td>
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<tr>
<td>D14S1426</td>
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<td>0.64</td>
</tr>
<tr>
<td>LPC</td>
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<td>D15S653</td>
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<td>D16S771</td>
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<tr>
<td>D16S3253</td>
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<td>D16S442</td>
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<tr>
<td>D18S844</td>
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<td>D20S898</td>
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</tr>
<tr>
<td>D20S477</td>
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<td>1.58</td>
</tr>
<tr>
<td>D21S1440</td>
<td>0.02</td>
<td>2.78</td>
</tr>
</tbody>
</table>

Boldface indicates suggestive linkage.

after accounting for simple linear effects of sex, age, and BMI. Thus, the polygenic model accounted for 45% of the total variation in PP. The full model, ie, the polygenic model plus the single locus components, gave a residual variance of 62.4. Thus, the full model accounted for 73% of the total variation in PP. The correlation between PP and SBP was 0.84, and the correlation between PP and DBP was 0.

Discussion

This is, to our knowledge, the first report of a genome scan for PP in a population-based sample. Given the strong correlation between PP and SBP, it is natural to ask whether genome scans of both traits found evidence for linkage in the same regions of the genome. For this data set, we have reported genome scans of SBP and DBP elsewhere. In the SBP genome scan, the 4 markers with the highest lod scores were the same as for PP reported in the present study. The lod scores for those 4 markers were 2.82 (D21S1440), 2.09 (D18S844), 1.64 (D8S1100), and 1.60 (D7S1799). Furthermore, 25 of the 31 markers that had lod scores >0.59 for PP also had lod scores >0.59 for SBP. The correlation between PP and SBP lod scores for those 25 markers was 0.92. This almost total overlap between PP and SBP is strong evidence that in this Mexican American population, PP and SBP have the same genetic etiology. This is surprising inasmuch as aortic stiffness is distinct from blood pressure and recent findings indicate that PP is a unique predictor of cardiovascular events. This may indicate that the uniqueness of PP is nongenetic. It should also be noted that PP might be too crude a measure of aortic stiffness to capture what is genetically unique about aortic stiffness.

In the model used for PP in the present study, we found significant sex-specific and genotype-specific effects of age, age<sup>2</sup>, and BMI on PP. Given the strong correlation between SBP and PP, these results can be regarded as confirming the result of Perusse et al,<sup>5</sup> who found sex-specific and genotype-specific effects of age on SBP.

Support for linkage of quantitative trait loci (QTL) affecting PP to the 4 regions located by the present study can be found in at least 1 independent study for each region. The suggestive linkage of a QTL affecting PP to chromosome 21 (D21S1440, z=2.78 and θ=0.02) is supported by evidence from 2 genome scans. Krushkal et al,<sup>6</sup> in a study of SBP, found a maximum lod score of 0.86 with a locus 3 cM away from D21S1440. Further support comes from a genome scan of DBP performed by Xu et al,<sup>7</sup> who found a maximum lod score of 2.20 with a locus 11 cM away from D21S1440.

The suggestive linkage of a QTL affecting PP to chromosome 7 (D7S1799, z=2.04 and θ=0.01) is supported by a genome scan of SBP reported by Rice et al.<sup>8</sup> They found a maximum lod score of 2.26 with a locus 20 cM from D7S1799.

The suggestive linkage of a QTL affecting PP to chromosome 8 (D8S1100, z=1.98 and θ=0.08) is supported by several studies. D8S1100 is 1.3 cM away from the aldosterone synthase gene (CYP11B2). A chimeric fusion of the aldosterone synthase gene and the adjacent 11β-hydroxylase gene (CYP11B1) is known to cause glucocorticoid-remediable aldosteronism, a rare monogenic form of hypertension.<sup>9</sup> Further evidence that variation at this candidate gene influences blood pressure variation comes from Davies et al,<sup>10</sup> who performed a case-control study of individuals from Glasgow, Scotland, in which polymorphisms in CYP11B2 were associated with hypertension and aldosterone excretion.

The suggestive linkage of a QTL affecting PP to chromosome 18 (D18S844, z=1.95 and θ=0.11) is supported by 2 studies. Pankow et al,<sup>11</sup> in a genome scan of the response of SBP to postural change, found a maximum lod score of 2.6 with a locus 27 cM away from D18S844. DeStefano et al,<sup>12</sup> in a study of orthostatic hypotension, found a significant maximum lod score of 3.21 with a locus 8.0 cM away from D18S844.

One of the 399 markers in the genome scan was a highly polymorphic marker at the angiotensinogen (AGT) locus. AGT is the most widely studied candidate gene for primary hypertension and has been linked to hypertension in this data set using hypertensive sibpairs.<sup>13</sup> AGT is therefore a natural
candidate for linkage to PP; however, in this analysis, AGT was excluded from linkage to PP ($\zeta = -9.94$ at $\theta = 0.00$). Thus, although AGT may have some effect in hypertensive subjects, it may not have a significant effect on the population.

In summary, we have found suggestive evidence for QTL that affects PP in 4 distinct chromosomal regions in a randomly ascertained Mexican American population. A comparison of genome scans indicates that PP and SBP are likely to have the same genetic etiology.

Acknowledgments

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References

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