Genome Scan Among Nigerians Linking Blood Pressure to Chromosomes 2, 3, and 19


Abstract—An understanding of the genetic influences on hypertension would help unravel the pathophysiology of this complex disorder and improve our understanding of causal mechanisms. Contemporary technology makes it possible to examine enough genetic markers to support a generalized search across the entire genome for candidate regions. In the present study, a family set was recruited from southwest Nigeria, and 378 microsatellite markers were typed on 792 individuals in 196 families. Multipoint variance component analysis identified linkage signals (logarithm of the odds [LOD]>1.74, \( P<0.0023 \)) for systolic blood pressure on 19p (D19S714) and 19q (D19S246), whereas for diastolic blood pressure, linkage was observed on 2p (D2S1790), 3p (D3S1304), 5q (D5S1462), 7p (D7S3046), 7q (D7S821), and 10q (D10S1221). Other regions of interest (1.18<LOD<1.74, 0.0023<\( P<0.01 \)) were found on chromosomes 1, 6, 8, 9, and 11. These results provide additional evidence of linkage between blood pressure and several genomic regions reported in previous studies. Some of these regions additionally harbor hypertension candidate genes. Although evidence of linkage for blood pressure has been very slow to accumulate, even in comparison to other complex traits, the sum of current evidence appears to implicate, in particular, 2p, 3p, and 19p. Study designs that make it possible to confirm these results with association analysis and narrow the genomic interval are needed in order to make progress in this field.

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Key Words: genes | blacks | blood pressure | chromosomes

Although blood pressure (BP) has long been known to be a heritable trait, the molecular basis for susceptibility to hypertension has been difficult to describe. The candidate gene approach should provide the greatest efficiency; however, the multiple physiological systems involved in BP regulation and the uncertainty about their causal role have thus far frustrated most attempts to define specific causal variants. Furthermore, although loci that explain a small proportion of the total variance have been identified, it is not known whether these loci should be considered typical of the genetic contribution to interindividual BP variation. A thorough search is required to be certain that the most influential regions have been identified. Genome-wide scan techniques have the advantage that they do not require prior knowledge of the loci involved, thereby posing an attractive alternative approach to candidate gene studies.

Unfortunately, genome-wide linkage studies suffer from low statistical power, and the published experience to date has led to inconsistent results for BP.1–12 With only 3 exceptions,5–7 these studies have not reported findings that met the original criteria for genome-wide significance suggested by Lander and Kruglyak.13 In addition to low statistical precision, an important limitation to studies of the genetics of hypertension is the constraints on the epidemiologic design. Human families are generally small; have a long generation time; and, when chronic disease is the trait of interest, may only express the phenotype in late adulthood. For BP in particular, the phenotype can often be unobservable because of treatment. Different geographic populations present contrasting advantages for the genetic studies of specific traits. To avoid many of the shortcomings outlined above, we conducted a population-based family survey in southwestern Nigeria. In addition to providing information of interest regarding one of the ancestral populations for African Americans, large families are common in this society and hypertension treatment is rare. In the present paper, we report findings from a genome-wide scan for BP among farmers from the Yoruba ethnic group.

Methods

Participant Recruitment

The sampling frame for this study was provided by the International Collaborative Study on Hypertension in Blacks (ICSHIB), as described in detail elsewhere.14–16 Nuclear families were identified on

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the basis of a middle-aged proband and his/her spouse, and all available first-degree relatives were enrolled. The family sets available at this phase of the study included 2259 persons. Individuals in larger families were chosen for genotyping. The most common pedigree type was father, mother, and ≥2 offspring. Of the 420 pedigrees, 360 included both parents. Half-sibs were encountered frequently, because of polygyny, and were enrolled whenever available. The protocol was approved by the institutional review board at Loyola University and by the ethics committee of the University College Hospital, Ibadan. Informed consent was presented in Yoruba or English and was obtained from participants by local staff.

Survey Methods

A screening examination was completed by trained research staff using a standardized protocol. Trained local interviewers obtained a medical history and a family pedigree in the participant’s native language. BP observers were trained and certified by a previously described procedure. Measurements were made in the sitting position, with the arm at heart after a 5-minute rest. An oscillometric device, previously evaluated in our field settings, was used for all BP measurements (Omron HEM-412). Three measurements were taken 3 minutes apart, and the average of the final 2 measurement were used in the analysis. Height was measured to the nearest 0.1 cm by use of a stadiometer consisting of a steel tape attached to a straight wall and a wooden headboard. The headboard was positioned with the participant shoeless, with feet and back against the wall and head held in the Frankfort horizontal plane. Body weight was measured to the nearest 0.2 kg by use of calibrated electronic scales uniform to all sites. Body mass index (BMI) was calculated as weight/height (kg/m²). Participants with hypertension were offered treatment after detection at the screening examination.

Genotyping

Genomic DNA was extracted and submitted to the National Heart, Lung, and Blood Institute (NHLBI) Mammalian Genotyping Service (Marshfield, Wis; http://research.marshfieldclinic.org/genetics). Tandem repeat markers were typed according the Marshfield “Set 9,” with an average map distance of 10 centimorgans (cM). During the preliminary analyses, it was determined that 3 markers were much more commonly associated with mendelian errors than were any of the others; it was inferred that genotyping errors were common, and those 3 markers were deleted. For the final linkage analyses, 378 markers were available.

Statistical Analysis

Both “Aspex” and “RELPAIR” were used to check the consistency of the pedigrees. Seven hundred ninety-two persons in 196 families were available in the analytic sample; 156 individuals were removed from the original sample because of pedigree inconsistencies or inadequate DNA specimens. After resolution of all instances of nonpaternity and other errors in the family structures, further mendelian errors were identified with “PedCheck” and linkage analysis programs. These errors were assumed to have occurred in the genotyping process, and the associated markers were set to zero among the appropriate family members.

BP was used as a continuous trait because none of the participants were receiving consistent treatment at the baseline examination. Heritability was estimated by use of the variance component method implemented in the SOLAR package when no specific locus effect was added, but was adjusted for sex, age, age², and BMI. Genomewide linkage analysis of SBP and DBP was performed by use of the multipoint variance component program in GENEHUNTER. The variance component method decomposes the total variance into the variances attributed to the additive effect of a quantitative trait locus, the polygenic effects, and random environmental effects. The likelihood ratio test was applied to test the null hypothesis of no additive genetic variance owing to the quantitative trait locus (QTL). Both full sibships and half sibships were used in the variance component method. Sex, age, age², and BMI were incorporated as covariates, and their effects were simultaneously estimated by the maximum-likelihood method. In all analyses, allele frequencies were estimated from the marker data. We used the Marshfield map distances in the linkage analysis. The multipoint identity by descent probabilities shared by a pair of relatives was calculated using all the marker genotype information at 1-cM intervals. Determining the significance of results of a genome scan remains a controversial topic. Substantial logarithm of the odds (LOD) scores (eg, >3.3) have generally been required to establish genome-wide significance. According to Rao and Province, however, a significance level of P=0.0023 (LOD=1.74) represents 1 false-positive per scan for a linkage study involving 400 makers. We used this latter criterion level to judge the significance of our linkage evidence. In addition, we conducted simulation to studies with our data set to compliment the calculation of LOD scores. In the simulation, we retained the pedigree structures, phenotypes, and covariates and simulated the marker data based on the observed allele frequencies. Because no single LOD score was obtained that reached genome-wide significance, we also estimated the probability of a false positive in the regions that appeared to replicate previous findings.

Results

The descriptive characteristics of the participants are presented in Table 1. The sample consisted of a total of 792 genotyped individuals in 196 families. Sex was not significantly associated with BP (P>0.59), whereas age, age², and BMI were. The residual after adjusting for sex, age, age², and BMI revealed minimal departure from normality, as measured by skewness (1.19 for SBP and 0.91 for DBP). The heritabilities for SBP and DBP after adjusting for the covariates sex, age, age², and BMI in this set of families were 0.35±0.08 and 0.38±0.07, respectively (P<10⁻⁴).

A search for linkage was performed across all 22 autosomes for BP, with sex, age, age², and BMI as covariates. Table 2 summarizes the principle findings of the multipoint linkage analysis for SBP and DBP. The complete genomewide scan results are displayed graphically in the Figure. We identified 9 chromosomal regions with a LOD score >1.18 for SBP; 2 of these regions had a LOD >1.74 (ie, 19p and 19q). Eleven regions were identified by the same criteria for

<table>
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<th>Variable</th>
<th>Mean±SD</th>
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<th>No.</th>
<th>Range</th>
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Table 1. Descriptive Characteristics of Participants and Families
DBP, with 6 having a LOD >1.74 (ie, 2p, 3p, 5q, 7p, 7q, and 10q). The largest single LOD score was 2.65 (P=0.00024), associated with SBP at the position 19q13.33.

We also conducted simulations to evaluate the statistical strength of our findings. At the genome-wide level, a LOD score >1.7 would have occurred >50% of the time in a study with the size and family structure of our sample, as would be anticipated given the number of separate tests that were carried out. We also focused simulations on the regions with previous linkage evidence where our LODs exceeded 1.7 (chromosome 2, region 94 to 114; chromosome 4, region 6 to 26; and chromosome 19, region 37 to 57). Based on 120 replications, we obtained only 1 to 3 LODs greater than the empirical values observed in those regions, suggesting that our results do provide evidence of replication for these specific regions.

**Discussion**

The primary goal of this study was to localize genomic regions that might affect variation in BP. Because it is a complex trait, influenced by multiple environmental and genetic factors and their interactions, BP has presented a particularly difficult challenge to genetic epidemiology. As is generally recognized, there are likely to be multiple QTLs involved in BP regulation in the general population, each with small-to-moderate effects. If, for example, the genetic component of BP variance is ≈ 35%, as noted here, and 7 genes of equal effect are segregating in the population, then each locus would have, on average, an effect in the range of 5%.

The study designs and sample sizes available for most investigations cannot reliably detect QTLs for BP in this range. Of course, genetic effects are likely to be unevenly distributed among loci, and specific genomic regions could be relatively more important in different populations.22 Given the uncertainty about these assumptions, we accepted the significance criteria of LOD >1.74, as recommended by Rao and Province.21 This criterion reduces the type II error but risks a higher false-positive rate, as demonstrated by our simulations. We therefore view the regions identified in this study as suggestive, as have others, requiring confirmation from other independent linkage analyses and from study designs with lower type II error rates. It is likely that more robust methods, such as well-designed association studies, will be required to define the loci that have sufficiently widespread impact to be of public health importance.22,23

Based on the argument outlined above, consistency across studies would be a more important standard against which to judge linkage results than would LOD scores from a single study.22,24 Accordingly, we draw attention to our findings on chromosomes 2 and 3. Linkage evidence was found with DBP on chromosome 2p12 near the marker D2S1790 (LOD=1.92). A relatively weaker result was also seen for SBP 20 cM away from D2S1790 (LOD=1.66). In the genome-wide study of the BP in Quebec families, Rice et al found LOD scores of 2.28 and 0.77 associated with marker D2S1790 for SBP and DBP, respectively. A LOD score of 3.92 was also identified to D2S1790 for SBP after adjusting for DBP in Mexican Americans.11 In a Chinese population, a LOD score of 2.2 was found in an adjacent region, and the HERITAGE Family Study recently reported a LOD score of 1.88 at 2p in African Americans.12 With postural BP change as the phenotype, Pankow et al reported linkage evidence in the same region, with a lower level of significance (LOD≈1.4). According to Lander and Kruglyak,13 linkage...
evidence with $P<0.01$ qualifies for consideration as a significant replication result after adjusting for multiple comparisons, and our result therefore meets this more conservative standard of evidence as well. Although we recognize that some of these findings do not completely overlap, it seems reasonable to conclude that studies in several populations have now yielded consistent linkage evidence in the region 2p11 to 2p12.3. The $\alpha_2$-adrenergic receptor gene, which is functionally implicated in BP regulation, is located less than 1 cM away from marker D2S1790; other candidates have been listed in the recent report by Rice et al.\textsuperscript{12} Combining the results of the present study with previous results, this region warrants further detailed investigation.

We also identified linkage evidence for DBP (LOD = 2.28) at the marker D3S1304 with a chromosomal location 3p26.2. SBP was linked to the same region (LOD = 1.43). Marker D3S2387, 3 cM away from D3S1304, was linked to SBP in a low-concordant sibpair study in a Chinese population (LOD = 2.03)\textsuperscript{2} and with SBP in the white sample in HERITAGE (LOD = 1.84).\textsuperscript{12} With the same logic as applied to chromosome 2, these data on 3p26.2 can be said to provide important linkage evidence to BP variation.

Our study also yields linkage evidence (LOD > 1.74) in several other regions. The LOD score of 2.65 on 19q13.33 was the strongest evidence of linkage observed in this study. In further close correspondence between our results and the HERITAGE study, a LOD score of 2.1 was observed in their African American population at 19p12.\textsuperscript{15} SBP also mapped to 19p13 in the earlier study by Rice et al.\textsuperscript{2} We did not find any evidence in the regions on chromosome 18 with significant linkage from deCODE\textsuperscript{7} or chromosome 17 from Framingham.\textsuperscript{8} Obviously, in this summary we have not attempted to provide a quantitative assessment of the published findings, and we acknowledge that as multiple reports for genome scans for BP accumulate, distinguishing replicate findings from mere coincidence will become a major statistical problem.\textsuperscript{25} Targeted linkage studies that pick high-priority regions ahead of time, thereby reducing the burden of multiple statistical testing, might be a particularly fruitful avenue to pursue at this juncture.

**Perspectives**

In conclusion, this African population sample of moderate size provides evidence in support of linkage on several chromosomal regions, 3 of which were identified in prior studies. Advantages of the analyses conducted in this study are the availability of relatively complete nuclear families and the absence of treatment for BP at the baseline screening examination. The regions of 2p and 3p warrant further examination. Additional linkage evidence would be helpful, and association studies using linkage disequilibrium mapping hold particular promise.\textsuperscript{23} With the demonstration that the genome is composed of pronounced haplotype blocks that are largely conserved across ethnic populations,\textsuperscript{6,27} a region-wide, or even genome-wide, association study may be feasible in the near future. Our recent analysis, based on the markers near ACE on chromosome 17 from the same Nigerian population, provides a practical demonstration of the increase in power that association studies have compared with linkage.\textsuperscript{28} Although very weak linkage evidence to BP was observed, significant association was found using multiple single nucleotide polymorphisms.\textsuperscript{28} Hence, well-designed association studies could be powerful tools in the next round of efforts to define the genetic underpinnings of hypertension.

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