Genome Scan for Hypertension in Nonobese African Americans

The National Heart, Lung, and Blood Institute Family Blood Pressure Program

Alanna C. Morrison, Richard Cooper, Steven Hunt, Cora E. Lewis, Amy Luke, Thomas H. Mosley, and Eric Boerwinkle

**Background:** Obesity is an important risk factor for hypertension, but epidemiologic studies provide evidence for the development of hypertension independent of obesity. In addition, the search for hypertension susceptibility genes should prove more informative when applied to a homogeneous subset of patients, such as those that are not obese. For this reason, we sought to identify genomic regions influencing susceptibility to hypertension in a nonobese sample of hypertensive African American families.

**Methods:** A genome-wide linkage scan was performed in a sample of 275 African American hypertensive families containing two or more nonobese (body mass index, $\leq 30$ kg/m$^2$) individuals recruited by Networks of the Family Blood Pressure Program (FBPP).

**Results:** The best evidence for linkage of hypertension among the FBPP African American families was found on chromosome 2 (log of the odds [LOD] = 3.59 at 230 cM). All other chromosomes contained LOD scores less than 2. The African American sibships from the GENOA Network appear to largely contribute to the evidence for linkage on chromosome 2 (LOD = 4.07 at 233 cM).

**Conclusions:** Significant evidence for linkage to hypertension in nonobese African American families was identified on chromosome 2q. These results suggest the presence of genes influencing susceptibility to adiposity-independent hypertension. Am J Hypertens 2004;17:834–838 © 2004 American Journal of Hypertension, Ltd.

**Key Words:** Hypertension, obesity, genetic linkage.
fluctuating susceptibility to adiposity-independent hypertension.

Methods

Study Population

Subjects in this study included 4404 individuals in 1819 African American pedigrees. These families were recruited by three of the four multicenter networks comprising the FBPP, a large NHLBI-funded study of the genetics of hypertension and its target organ complications. Detailed descriptions of the individual Networks with African American participants (GenNet, HyperGEN, and GENOA) and hypertension diagnostic criteria are available elsewhere. Briefly, GenNet sibships were ascertained on the basis of the proband having an elevated BP (>80th percentile for age/sex) and one or more siblings available for study regardless of BP level or hypertension status. Parents of the siblings were also collected when available. HyperGEN and GENOA recruited sibships through probands with diagnosed hypertension. At each field center, BP was measured using an identical protocol and a standardized algorithm was used to define the BP diagnostic categories. Hypertension was defined as a systolic BP >140 mm Hg, a diastolic BP >90 mm Hg, or regular use of antihypertensive medication. Participants were genotyped by the Mammalian Genotyping Service (MGS) in Marshfield, Wisconsin, using the same set of 387 highly polymorphic microsatellite markers with an average intermarker distance of 10 cM. Each study has been approved by the appropriate local institutional review board and all participants provided written informed consent.

Of the 1819 African American pedigrees, 964 were informative for this analysis in that the families contained two or more hypertensive siblings available for nonparametric linkage analysis. The number of contributing individuals and pedigrees by Network is detailed in Table 1. For this study we were interested in hypertensive sibpairs that also shared a phenotype of nonobesity defined by a body mass index (BMI) ≤30 kg/m². Any individual with a BMI greater than 30 kg/m² had their hypertension status set to missing to maintain full family structure while allowing for only nonparametric linkage analysis of hypertensive sibpairs concordant for nonobesity. This resulted in a total of 275 informative families.

Linkage Analyses

Multipoint nonparametric linkage analysis was performed using Merlin. Marker order and map locations were deduced from the Marshfield map (Marshfield Clinic, Marshfield, WI). Allele frequencies were estimated by allele counting among all individuals. Linkage evidence is expressed in terms of log of the odds (LOD) scores. A genome scan was performed for the entire sample of 275 informative pedigrees from the FBPP. Upon identification of a LOD ≥3, Network-specific linkage analysis on that chromosome was evaluated.

Results

The results from the linkage analysis of nonobese hypertensive FBPP families are reported as multipoint LOD score plots for chromosomes containing a peak LOD ≥3.0. Only chromosome 2 met this criterion with a LOD of 3.59 at 230 cM (Fig. 1). Table 2 details the maximum peak LOD scores and their respective chromosomal position for each autosome. When Network-specific linkage on chromosome 2 was evaluated (Fig. 1), the best evidence for linkage was found in only one Network, GENOA (LOD = 4.07 at 233 cM). The other two Networks, GenNet and HyperGEN, had minimal evidence for linkage (LOD ≤1) within the interval defined by the linkage peak identified in the total sample of FBPP families. The maximum peak LOD scores for GenNet and HyperGEN on chromosome 2 occur, respectively, at 184 cM (LOD = 1.11) and 64 cM (LOD = 0.58).

Given the significant evidence for linkage in the GENOA nonobese hypertensive sibpairs and to further test whether the identified region suggests the presence of genes influencing susceptibility to adiposity-independent hypertension, linkage on chromosome 2 was evaluated in GENOA hypertensive sibpairs that also shared a phenotype of obesity defined by a BMI >30 kg/m². Any individual with a BMI ≤30 kg/m² had their hypertension status set to missing to maintain full family structure while
allowing for only nonparametric linkage analysis of GENOA hypertensive sibpairs concordant for obesity. This resulted in a total of 178 informative families. A LOD score of 0.33 was shown at 233 cM, the chromosomal location of the peak evidence for linkage in the GENOA nonobese hypertensive sibpairs (Fig. 1). The maximum peak LOD score for the GENOA obese hypertensive sibpairs occurs at 90 cM (LOD = 1.32).

Discussion
The known familial aggregation of BP and hypertension is attributable to shared genetic factors. However, the search for hypertension susceptibility genes is made difficult because of underlying genetic heterogeneity of the phenotype and the likely small effects of any single genetic variation. Minimizing heterogeneity among families through the analysis of appropriate groups may increase success in the identification of suggestive genomic regions. In this study, we define groups of individuals of sufficient size and homogeneity by identification of hypertensive sibpairs with a BMI \( \geq 30 \) kg/m\(^2\). Significant evidence for linkage to hypertension in nonobese African American families from the FBPP was identified on chromosome 2 at 230 cM (LOD = 3.59). These results suggest the presence of genes influencing susceptibility to adiposity-independent hypertension.

Support for a hypertension candidate gene on chromosome 2 comes from the fact that the reported results meet the genome-wide criteria for suggestive evidence of linkage\(^9\) as well as corroborating evidence from published studies of linkage to BP phenotypes in the region. Interestingly, the published studies with consistent evidence of linkage in the chromosome 2q region involved samples of individuals ascertained with regard to body size phenotypes, either by design or by the nature of the study population. For example, linkage analysis of BP Old Order Amish indicated evidence on chromosome 2q for a quantitative locus influencing diastolic (LOD = 3.36 at 217 cM) and systolic BP (LOD = 1.64 at 221 cM).\(^{10}\) Hsueh et al\(^{10}\) report that the Old Order Amish are a rural-living population characterized by their eschewal of technological innovation, and the mean BMI for women (28.0 ± 5.7 kg/m\(^2\)) and men (26.2 ± 3.8 kg/m\(^2\)) in this study was less than 30 kg/m\(^2\). An affected sibpair linkage study was performed in a sample of families identified through the Finnish Twin Cohort Study that was selected on the basis

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<tr>
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<tr>
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Chromosomes 9, 15, 16, 20, 21, and 22 did not demonstrate any evidence of linkage to a hypertension susceptibility gene in this sample.

FIG. 1. Linkage results for chromosome 2. Black diamonds are the total sample of Family Blood Pressure Program nonobese hypertensive families. Black triangles are the GENOA nonobese hypertensive families. Open triangles are the GENOA obese hypertensive families. Grey squares are the GenNet nonobese hypertensive families. Grey circles are the HyperGEN nonobese hypertensive families.
of early-onset hypertension with minimal presence of other phenotypic risk factors such as obesity.\textsuperscript{11} Some evidence for linkage was also detected on chromosome 2q (LOD = 2.96). Finally, the Quebec Family Heart study performed a genome-wide linkage analysis for systolic and diastolic BP among randomly ascertained and obese pedigrees.\textsuperscript{12} Consistent with our results of adiposity-independent linkage on chromosome 2q, linkage for systolic or diastolic BP was not evident among obese pedigrees for the region implicated in our study.

Given the significant evidence for linkage on chromosome 2q in the sample of nonobese hypertensive African American FBPP families, Network-specific linkage was evaluated and the best evidence for linkage was found in GENOA (LOD = 4.07 at 233 cM). GenNet and HyperGEN had minimal evidence for linkage (LOD \textless 1) within the interval defined by the linkage peak identified in the total sample of FBPP families. Lack of evidence of linkage in GenNet is likely due to issues of power. Only 12 GenNet families were informative for this analysis (Table 1) in that they contained two or more nonobese hypertensive siblings available for nonparametric linkage analysis. The low number of hypertensive siblings available in GenNet is because the ascertainment scheme involved selection of young probands with elevated, yet normal BP. The number of informative HyperGEN families is of the same order of magnitude as those identified in GENOA, and the two Networks shared similar ascertainment schemes. Distribution of sibship size, mean BMI, and the proportion of hypertensive individuals that were nonobese in the informative families did not differ significantly between HyperGEN and GENOA (data not shown). The discrepancy in the observed linkage results between these two Networks may be due to true differences in genetic effects of loci across the different pedigrees in the region of chromosome 2q. A simulation study of polygenic disease models suggests that individual loci may have such a small population-wide effect on susceptibility that they are difficult to detect consistently without very large samples.\textsuperscript{13}

Epidemiologic and clinical studies\textsuperscript{1,2} indicate that sympathetic nervous system (SNS) activity plays a role in hypertension among obese individuals. However, other factors may play a role in adiposity-independent hypertension. For these reasons we were interested in the identification of potential candidate genes in the linked region on chromosome 2q. This region overlaps with a region detected in two genome-wide scans for familial primary pulmonary hypertension (PPH).\textsuperscript{14,15} A rare disease characterized by elevated pulmonary artery pressure. The putative trait locus in this region is BMPR2, encoding a TGF-\(\beta\) type II receptor.\textsuperscript{16} The TGF-\(\beta\) signaling pathway plays a role in the maintenance of blood vessel integrity; hence, it is possible that other defects in BMPR2 may produce a common hypertensive phenotype by affecting systemic endothelial vasculature or function. Additional genes within this region potentially playing a role in adiposity-independent hypertension through pathways influencing vascular wall function include the parathyroid receptor 2 and insulin growth factor binding proteins 2 and 5. This region also contains a cluster of immunoglobulin superfamily genes that encode the integrin subunits. The \(\alpha\) and \(\beta\) integrin subunits combine to form heterodimeric signaling molecules involved in a wide variety of physiologic processes, including angiogenesis, immune regulation, and hemostasis.\textsuperscript{17}

In conclusion, these data reflect the utility of taking advantage of methods involving a subset of individuals of sufficient sample size and homogeneity in the analysis of complex traits. Identification of genomic regions demonstrating linkage for a complex disease, taking into account information from a related trait, is a powerful method to better understand the underlying mechanisms of the disease and their relationships to the trait. Future analyses of complex diseases will benefit from the development and application of analytical methods that have the ability to systematically evaluate the contribution of genes operating in heterogeneous environments without unduly sacrificing sample size or power.

**Acknowledgments**

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**References**