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Meta-Analysis of Genome-Wide Scans for Blood Pressure in African American and Nigerian Samples

The National Heart, Lung, and Blood Institute GeneLink Project

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Background: In many genetic studies of complex traits, sample sizes are often too small to detect linkages of low-to-moderate effects. However, the combined linkage evidence across several studies can be synthesized using meta-analysis with the aim of providing more definitive support of linkage.

Methods: In the current study using the National Heart, Lung, and Blood Institute (NHLBI) GeneLink Project, a meta-analysis based on a modification of Fisher's method of pooling *P* values was used to investigate linkage for systolic blood pressure (SBP) and diastolic blood pressure (DBP) values across three studies involving African American and Nigerian families (HyperGEN, Health, Risk Factors, Exercise Training and Genetics [HERITAGE], and Genetics of Hypertension in Blacks).

Results: The meta results suggest two regions (2p and 7p) provide enhanced linkage evidence compared with the individual study results. The maximal meta Lod score of 2.9 on 2p14-p13.1 (64–78 cM) represented ~1-Lod unit increase over the respective individual study scores. This general region has been implicated previously involving primarily families of white ethnicity and provides confirmatory evidence that this QTL is common across ethnic groups. The second finding at 7p21.3-p15.3 (8–25 cM) provided a meta Lod of 3.5. Although region was implicated primarily in the Nigerian subjects the low-level but consistent support involving the African American families (individual Lod score of 1.0) suggests a novel QTL with respect to BP variation in individuals of black ethnicity.

Conclusions: Follow-up studies involving positional cloning efforts of the combined families showing linkage evidence in these regions (particularly 2p) may be warranted to verify these findings and identify the genes and causative variants. Am J Hypertens 2006;19:270–274

Key Words: Linkage, 2p14-2p13.1, 7p21.1-7p15.3, hypertension, African American.

Mapping the genes involved in complex traits such as hypertension has proved to be difficult, as evidenced by the limited replication of findings across studies. Although this could be caused by different underlying physiologic, environmental, and genetic determinants among different ethnic or risk groups, it could also be a function of low power among the individual studies to detect these small-to-moderate effects. Consequently, meta-analytic strategies that simultaneously assess the linkage evidence across multiple studies may provide more definitive evidence of genetic linkage for hypertension, as has been suggested in two recent reports.[1,2] Koivukoski et.[2] analyzed genome-wide results from nine published studies involving populations of white ethnicity

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and reported that the modest to nonsignificant results in the individual studies produced significant and highly suggestive meta-analysis linkage on 3p14.1-q12.3 and on 2p12-q22. Another meta-analysis involved four multicenter networks forming the Family Blood Pressure Program (FBPP) and incorporated three ethnic groups (white, African American, and Asian).[1] In that report no region showed striking evidence, although several small peaks (including chromosome 2p) were noted. In the current investigation, a genome-wide meta-analysis linkage approach is used to evaluate the evidence across three populations of black ethnicity participating in the GeneLink project (http://genelink.nhlbi.nih.gov/index.jsp) sponsored by the National Heart, Lung, and Blood Institute. Two of the genome-wide scans involve African-American families and the third is based on Nigerian families of black ethnicity.

Methods

Study Populations

The details of each individual genome scan analysis have previously been presented in detail.[3–5] Table 1 represents only a brief summary of the African-Americans from the HERITAGE and HyperGEN studies and Nigerians from the Genetics of Hypertension in Blacks study. Although the HERITAGE and Nigerian samples consist of nuclear families or pedigrees that were selected randomly with regard to hypertension status, the HyperGEN study consisted entirely of sibships selected for moderate to severe hypertension. Previous linkage analyses for each study were published using multipoint variance component methods as outlined in Table 2. The blood pressure (BP) measures were similarly adjusted across studies and accounted for the effects of age, sex, and adiposity. The HyperGEN study also adjusted for the effects of medication use, whereas individuals on hypertensive medications were excluded from the HERITAGE and Nigerian samples.

Meta-Analysis

The meta-analysis was conducted by combining *P* values from the individual scans. As described by Province et al.,[1] Fisher's technique was used to combine *P* values with a modification to correct for bias in pooling nonparametric LOD scores that were exactly zero.[6] This modified Fisher's method was applied at every 1-cM location to produce meta–*P* values across the entire genome scan. The meta–*P* values were then converted back to LOD scores for a graphical representation of the results, as there is a direct correspondence between *P* values and LOD scores.[7]

Results

Figure 1 provides the genome-wide scan results for the meta-analysis and for each individual study. As shown, there are two regions (on chromosomes 2 and 7) where the

Table 1. Description of sample

Sample	Selection/Structure	Group	Age	Age Range	SBP ± SD	DBP ± SD	BMI ± SD	Fams	Indivs	Sib Pairs
Nigerian	Random/Pedigrees	All	40.7 ± 19.7	13–90	126.7 ± 27.3	76.6 ± 17.7	21.1 ± 4.1	196	792	259
HERITAGE	Random/nuclear families	F	50.0 ± 7.2	<66	126.7 ± 12.8	76.6 ± 9.2	27.5 ± 5.2	125	29	136
		M	46.5 ± 6.7		129.0 ± 13.7	77.8 ± 8.2	29.3 ± 5.3		58	
		S	27.2 ± 7.2	>17	125.2 ± 8.9	71.4 ± 7.1	27.4 ± 5.8		84	
		D	27.8 ± 7.5		119.8 ± 11.7	70.8 ± 8.2	28.0 ± 7.0		146	
HyperGen	HT sibs	All	47.2 ± 13.0	18–85	130.4 ± 22.3	74.7 ± 11.7	32.1 ± 7.5	1208	2401	1663

BMI = body mass index; D = daughters; DBP = diastolic blood pressure; F = fathers; Fams = families; HERITAGE (HEalth, RIsk factors, exercise Training And GEnetics) and HyperGen were African-American samples; HT = hypertensive; Indivs = individuals; M = mothers; Nigerian sample = Genetics of Hypertension in Blacks study; S = sons; SBP = systolic blood pressure; Sib pairs = sibling pairs.

Table 2. Descriptions of methods in original studies

Sample	Marker/Map Set	Average Intermarker Distance	No. of Markers	Adjustments	Linkage Method	IBD
Nigerian	Marshfield Set 9	10cM	378	Age, age^2, BMI, sex	GENEHUNTER VC	Multipoint
HERITAGE	LDB (Southampton)	7cM	509	Age, age^2, age^3, BMI, sex	SEGPATH VC	Multipoint
HyperGen	Marshfield Set 9	10cM	387	Age, age^2, BMI, sex, Meds*	SEGLINK VC	Multipoint

BMI = body mass index; VC = variance component.
* Model developed on random sample and parameter values applied to hypertensive sibships.

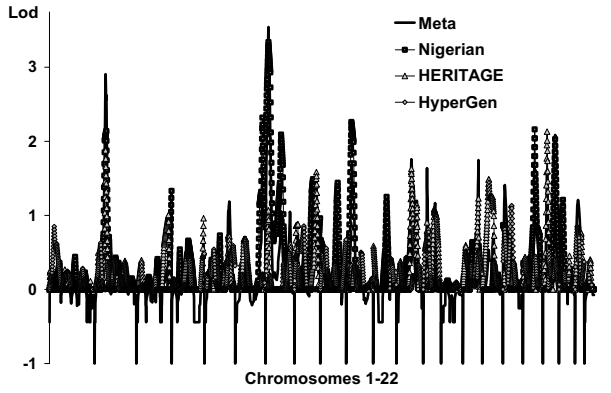

FIG 1. Meta and individual study Lod scores for systolic blood pressure (SBP) genome scan across 22 autosomes.

meta Lod score provides enhanced linkage evidence as compared with the individual scans. Figure 2A (chromosome 2) and Fig. 2b (chromosome 7) depict these results. The maximal meta Lod score on chromosome 2p was 2.91 at 69 cM, and on chromosome 7p the maximal meta Lod

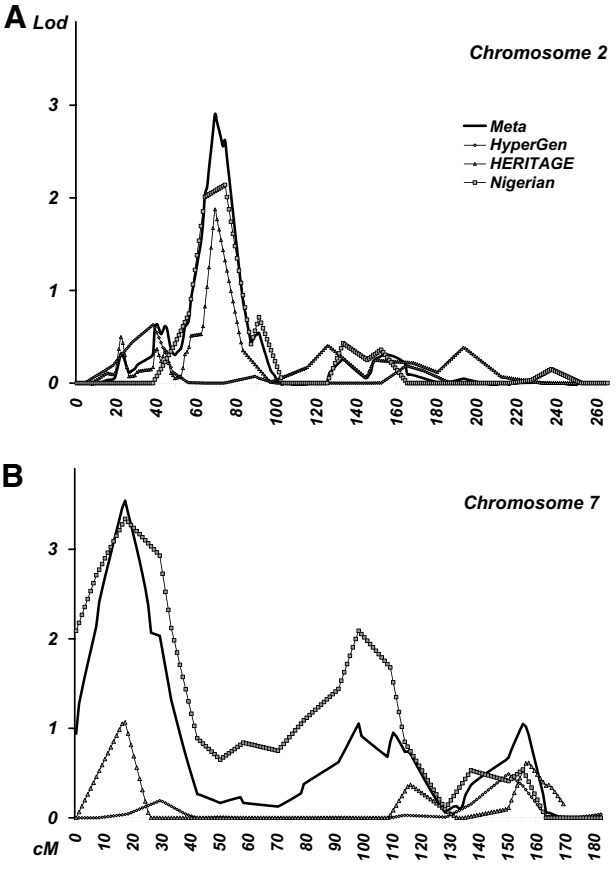

FIG 2. Meta-analysis Lod scores for SBP genome scans for chromosomes 2 (**top panel, a**) and 7 (**bottom panel, b**). Chromosome 2 region peaks at 69 cM (1-Lod interval 64–78 cM, 2p14–2p13.1). Lod scores for meta-analysis and for Nigerian and HERITAGE samples at 69 cM are 2.91, 2.08, and 1.88, respectively. Chromosome 7 region peaks at 17 cM (1-Lod interval 8-25 cM, 7p22.17p15.3). Lod scores for Meta analysis, Nigerian, and HERITAGE samples at 17 cM are 3.54, 3.34 and 1.07, respectively.

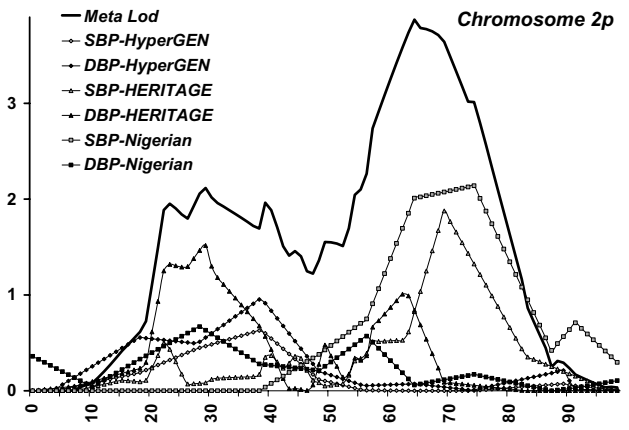

FIG. 3. Detail of chromosome 2p region showing meta-analysis results that include all phenotypes. Systolic blood pressure (SBP) region peaks at 64 cM. The Lod score for meta-analysis is 3.9, Nigerian, and HERITAGE SBP maximal Lods in the region are 2.09 and 1.9, and HyperGEN HT status maximal Lod is 2.0. A secondary peak is seen about 30 cM upstream at 29 cM (1-Lod interval 21–47 cM) for diastolic blood pressure (DBP phenotypes. Meta, HERITAGE, and Nigerian DBP Lods are 2.1, 1.5, and 0.67, respectively.

score was 3.54 at 17 cM. When all phenotypes (systolic BP [SBP] and diastolic BP [DBP] as well as a qualitative trait for hypertension (HT) status in HyperGEN) were included in the analysis, the maximal meta Lod on 2p was 3.9 at 64 cM, as shown in Fig. 3. Although this QTL appears specific to SBP and HT status, a secondary peak about 30 cM upstream (at 29 cM) was noted primarily for DBP.

Discussion

A meta-analysis of genome-wide linkage scan results from more than 3500 individuals in more than 1500 families of black ethnicity was carried out. Few exciting results were originally reported in the separate scans. However, the pooled linkage information indicates there are two genomic regions having consistent effects across samples on chromosomes 2p14-2p13.1 and 7p21.3-7p15.3. Although the 2p (meta Lod of 2.9) finding represents a replication of several other reports, the 7p result appears to be novel.

The finding on 2p (64-78 cM) has been reported at moderate levels in multiple studies. For example, Samani[8] reports that of 20 genome-wide scans reviewed, six of them[4,5,9–12] show a signal on 2p. These samples vary considerably with regard to selection criteria, phenotype, and ethnic background, and the linkage region is quite broad (26 to 115 cM), suggesting various interpretations such as multiple genes, false positives, or map differences. Questions concerning the broadness/multiplicity of this peak were followed-up in the current dataset by including all available phenotypes in the hypertension domain (systolic and DBP and hypertension status). Two distinct regions were noted (Fig. 3), one primarily for SBP and hypertension status (57–76 cM, maximal Meta Lod = 3.9) and another primarily for DBP (20–47 cM, maximal Meta Lod = 2.12). Although the meta Lod scores in this case are biased because of inclusion of the same families across multiple phenotypes, these results clearly suggest two nonoverlapping regions and consequently two inferred QTLs.

Of more than 80 genes mapped to the region on 2p14-p13.1 (64–78 cM), several have been linked or associated with hypertension or BP variation or both. For example, adducin (ADD2, 70 cM) produces a protein that is associated with membrane ion transport, and certain polymorphisms play a role in BP variation in Milan hypertensive rats,[13] who display dysfunctions also seen in human beings with primary hypertension. Also in this region is a novel G-protein–coupled receptor (GPR723 at 68 cM) that is closely related to the Y-receptor family for neuropeptide Y (NPY), peptide YY (PYY), and pancreatic polypeptide (PP), which mediate several physiologic effects including regulation of BP.[14] Angius et al.[12] also reported linkage at 2p25-p24 (HYT4) in Chinese families with essential hypertension. However, this QTL is 60 cM upstream of the 2p14-p13.1 region and less than 20 cM upstream of the secondary peak noted on 2p in this report. Other known genes in this region (eg, calcineurin B (PPP3R1) and an anticoagulant (ANXA4) play roles in BP regulation although no linkages or associations with BP or hypertension were found.

The second finding from the current study was on 7p (8–25 cM, Meta Lod 3.5). Although this meta signal was primarily attributed to the Nigerian sample (Lod 3.3), there was a clear and supporting but nominal peak (Lod = 1.1) for the HERITAGE African American sample at this same location. None of the previous 20 studies reviewed by Samani[8] reported signals in this region, although linkage with hypertension in Utah pedigrees was noted about 40 cM downstream.[15] Of the many known genes in this region, to date no reports of linkage or association with BP variation or hypertension were found. Although not shown on the graph of chromosome 7, DBP in both the Nigerian and HERITAGE samples also peaks at this same location, although the individual Lod scores are about 1.0. Together these results indicate a possible novel QTL for BP variation in this 7p region.

In summary, these meta-analyses provide clear indications that pooling the evidence across multiple linkage scans can produce positive results. This appears to be particularly important when the individual studies tend to be based on small samples and thus have low power to detect moderate effects. This is a problem for many studies of particular ethnic groups such as African American families in which paradoxically the prevalence of hypertension is typically higher compared with that in the general population. Two findings were noted in this meta-analysis of African American and Nigerian black individuals. For one (on 2p), the result provides additional replication of findings already noted in other studies of individuals of primarily white ethnicity. However, in the

current study the meta-analysis provides additional evidence that there are likely two QTLs in this region, one primarily for SBP and hypertension and the other for DBP. For the other result on 7p the meta-analysis provided confirmatory evidence of the signal noted previously only in the Nigerian sample, supporting the notion that a novel QTL for BP variation in families of black ethnicity may reside here. Finally, although few of the original individual results were outstanding by themselves and could be caused by false-positive peaks, the pooled evidence supports the notion that these consistencies may be indicative of true signals. Given the complex nature of the hypertension phenotype and the expected modest effects that each of several contributory genes will have on the trait at the population level, it is unlikely that any single study will have adequate power to definitively identify the QTLs. Meta analysis and resources such as the National Heart, Lung, and Blood Institute GeneLink Project provide a method for synthesizing the combined evidence to eventually locate these elusive genes.

References

1. Province MA, Kardia SLR, Ranade K, Rao DC, Thiel BA, Cooper RS, Risch N, Turner ST, Cox DR, Hunt SC, Weder AB, Boerwinkle E: A meta-analysis of genome-wide linkage scans for hypertension: The National Heart, Lung, and Blood Institute Family Blood Pressure Program. Am J Hypertens 2003;16:144–147.
2. Koivukoski L, Fisher SA, Kanninen T, Lewis CM, von Wowern F, Hunt S, Kardia SLR, Levy D, Perola M, Rankinen T, Rao DC, Rice T, Thiel BA, Melander O: Meta-analysis of genome-wide scans for hypertension and blood pressure in Caucasians shows evidence of susceptibility regions on chromosomes 2 and 3. Hum Mol Genet 2004;13:2325–2332.
3. Cooper RS, Luke Am Zhu X, Kan D, Adeyemo A, Rotimi C, Bouzekri N, Ward R: Genome scan among Nigerians linking blood pressure to chromosomes 2, 3, and 19. Hypertension 2002;40:629–633.
4. Rice T, Rankinen T, Chagnon YC, Province MA, Perusse L, Leon AS, Skinner JS, Wilmore JH, Bouchard C, Rao DC: Genomewide linkage scan of resting blood pressure: HERITAGE Family Study. Hypertension 2002;39:1037–1043.
5. Rao DC, Province MA, Leppert MF, Oberman A, Heiss G, Ellison RC, Arnett DK, Eckfeldt JH, Schwander K, Mockrin SC, Hunt SC: A genome-wide affected sibpair linkage analysis of hypertension: the HyperGEN network. Am J Hypertens 2003;16:148–150.
6. Province MA: The significance of not finding a gene. Am J Hum Genet 2001;69:660–663.
7. Ott J: Analysis of Human Genetic Linkage. Johns Hopkins University Press, Baltimore, 1991, pp 1–302.
8. Samani NJ: Genome scans for hypertension and blood pressure regulation. Am J Hypertens 2003;16:167–171.
9. Krushkal J, Ferrell R, Mockrin SC, Turner ST, Sing CF, Boerwinkle E: Genome-wide linkage analyses of systolic blood pressure using highly discordant siblings. Circulation 1999;99:1407–1410.
10. Rice T, Rankinen T, Province MA, Chagnon YC, Perusse L, Borecki IB, Bouchard C, Rao DC: Genome-wide linkage analysis of systolic and diastolic blood pressure: the Quebec Family Study. Circulation 2000;102:1956–1963.
11. Atwood LD, Samollow PB, Hixson JE, Stern MP, MacCluer JW: Genome-wide linkage analysis of blood pressure in Mexican Americans. Genet Epidemiol 2001;20:373–382.
12. Angius A, Petretto E, Maestrale GB, Forabosco P, Casu G, Piras D, Fanciulli M, Falchi M, Melis PM, Palermo M, Pirastu M: A new essential hypertension susceptibility locus on chromosome 2p24–p25 detected by genomewide search. Am J Hum Genet 2002;71:893–905.
13. Bianchi G, Tripodi G, Casari G, Salardi S, Barber BR, Garcia R, Leoni P, Torielli L, Cusi D, Ferrandi M, Pinna LA, Baralle FE, Ferrari P: Two point mutations within the adducin genes are involved in blood pressure variation. Proc Natl Acad Sci USA 1994;91:3999–4003.
14. Parker R, Liu M, Eyre HJ, Copeland NG, Gilbert DJ, Crawford J, Sutherland GR, Jenkins NA, Herzog H: Y-receptor-like genes GPR72 and GPR73: Molecular cloning, genomic organization and assignment to human chromosome 11q21.1 and 2p14 and mouse chromosome 9 and 6. Biochim Biophys Acta 2000;1491:369–375.
15. Hunt SC, Ellison RC, Atwood LD, Pankow JS, Province MA, Leppert MF: Genome scans for blood pressure and hypertension: the National Heart, Lung, and Blood Institute Family Heart Study. Hypertension 2002;40:1–6.