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NOVEL GENETIC VARIANTS CONTRIBUTING TO LEFT VENTRICULAR HYPERTROPHY: THE HYPERGEN STUDY:

Genetic variants for LV hypertrophy

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Abstract

Objectives—To identify genes contributing to variation in echocardiographic left ventricular (LV) mass and related traits using linkage and linkage disequilibrium analysis in sibships ascertained on hypertension.

Methods—The HyperGEN Study of LV hypertrophy characterized LV mass, relative wall thickness (RWT), and aortic root diameter (ARD) with echocardiograms collected using a standardized protocol at four HyperGEN field centers. A high-throughput scanning fluorescence detector system genotyped 387 polymorphisms distributed throughout the genome. Linkage analyses were conducted once genotyping results became available for 885 siblings from 382 sibships.

Results—Although single LOD score peaks ≥ 1.2 were found on chromosomes 1, 4, 5, 6, 7, 8, 9, 10, 12, 14, 17, and 21, we observed a broad band of peaks in both ethnic groups (white and black) on chromosome 4 and selected candidate genes (NPY1R, NPY2R, NPY5R, SFPR2, CPE, IL15, EDNRA) from this region. Using cases and controls from extremes of the LV mass index, RWT, and ARD distributions, we assessed associations with these phenotypes and haplotype-tagging single nucleotide polymorphisms (SNPs) in the candidates. Among blacks, SNPs in IL15, NPY2R, and NPY5R showed strong evidence for association ($p < 0.005$); all candidates except EDNRA showed suggestive association ($p < 0.05$). In whites, NPY2R, NPY5R, and SFRP2 SNPs offered suggestive evidence of association with one or more traits ($p < 0.05$).

Conclusions—Genetic variation in NPY1R, NPY2R, NPY5R, CPE, IL15, and SFRP2, detected using linkage analysis in hypertensive siblings, was associated with LV phenotypes in blacks and/or whites.

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Conflicts of interest
All authors report no conflicts of interest.

Keywords

left ventricular hypertrophy; genetics; echocardiography

Introduction

Increased mass of the left ventricle maintains cardiac output in response to pathological stimuli such as hypertension, obesity, and myocardial injury [1]. Left ventricular (LV) mass is a sensitive predictor of cardiovascular mortality and morbidity in all genders, races, and ages [2-4]. In addition to increased LV mass, abnormal LV geometry (i.e., relative wall thickness) is a further predictor of adverse cardiovascular events [5]. Echocardiographic measurements of aortic root diameter (ARD) also predict cardiovascular morbidity and mortality [6].

Evidence suggests echocardiographically-derived structural characteristics are, in part, under genetic control, with heritability estimates falling between 0.08 and 0.69 [7,8]. The normal population distribution of LV mass suggests that this phenotype is a complex trait influenced by multiple genes. Association studies in human populations, viewed *in toto*, support this inference. Polymorphisms of candidate genes on different chromosomes in diverse pathways (e.g., the angiotensin converting enzyme (ACE) [9], major histocompatibility complex [10], guanine nucleotide-binding protein (GNB3) [11], insulin-like growth factor (IGF-1) [12], and neuropeptide Y (NPY) genes [13]) have been associated with LV mass. However, other studies have failed to replicate the association with some of these polymorphisms (e.g., ACE [14], GNB3 [15]). Evidence of association of other echocardiographic measures of LV geometry with various polymorphisms is nearly equally abundant and similarly contradictory [16,17]. Aortic root diameter has been the subject of fewer genetic studies; however, aortic root dilation is characteristic of Marfan Syndrome, an uncommon autosomal dominant disorder involving mutations in the fibrillin-1 gene on chromosome 15.

The identification of genes that predispose hypertensive individuals to abnormal LV structural parameters may offer strategies for improved prognosis, prevention, and treatment of LV hypertrophy. Discovery of these genes remains a priority given the increasing prevalence of heart failure [18] and the documented association of LV hypertrophy and dysfunction with increased risk of heart failure [19]. The Hypertension Genetic Epidemiology Network (HyperGEN) study of LV hypertrophy was designed to detect genetic loci contributing to LV mass and related LV phenotypes using linkage in sibships ascertained on hypertension. Here we summarize linkage and association results for biologically relevant positional candidate genes in linked regions from linkage studies for several echocardiographic structural phenotypes. For the candidate genes we identified, we verified haplotype-tagging sequence variations and genotyped them in an independent group of HyperGEN participants. We demonstrate herein that the strategy of linkage-based approaches remains a valuable tool for dissection of complex diseases, and we provide evidence for novel positional candidate genes that contribute to echocardiographic structural abnormalities among hypertensive subjects.

Methods

Study population

Study participants were recruited from the Family Blood Pressure Program's Hypertension Genetic Epidemiology Network (HyperGEN) [20]. HyperGEN is one of four networks sponsored by the National Heart, Lung and Blood Institute to identify genetic contributions to hypertension. Subjects were drawn from population-based cohorts (from the

Atherosclerosis Risk in Communities Study in Minneapolis, MN and Forsyth County, NC; the Minnesota Heart Survey; and the Utah Health Family Tree Study) or from the community-at-large (Birmingham, AL). HyperGEN recruitment criteria required that participating sibships had ≥ 2 siblings who had been diagnosed with hypertension before age 60. Hypertension was defined as current antihypertensive treatment or an average systolic blood pressure (BP) ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg at two separate clinic visits. Average BP was calculated using the second and third measurements of three readings using an oscillometric BP monitor (Dinamap 1846 SX/P; GE Healthcare, Waukesha, WI). Individuals with a history of type 1 diabetes or severe renal disease were excluded. The HyperGEN population is described more completely elsewhere [20]. The linkage analyses presented here was conducted on the 885 HyperGEN participants with data available from the initial round of genotyping. This study was approved by the centers' institutional review boards, and all subjects gave informed consent.

Echocardiographic measures

Doppler, two-dimensional (2D) and M-mode (2D-guided) echocardiograms were performed following a standardized protocol previously described [21]. Certified sonographers from each field center were trained at the echocardiography reading center (New York Hospital-Cornell Medical Center). M-mode and 2D echocardiograms via the parasternal acoustic window were recorded for ≥ 10 beats. Measurements were made at the echocardiography reading center using a computerized review station equipped with a digitizing tablet and monitor overlay used for calibration and quantification (Digisonics, Inc., Houston, Texas). LV internal end-diastolic diameter and posterior wall thickness were measured by M-mode echocardiography according to American Society of Echocardiography (ASE) recommendations [22]. When the M-mode beam was not optimally aligned, 2D linear measurements were made according to ASE recommendations [22]. Relative wall thickness was calculated as RWT = 2PWT/LVID. LV mass was calculated using end-diastolic dimensions by an anatomically validated formula [23], and indexed to height$^{2.7}$ (hereafter left ventricular mass index or LVMI). Reproducibility was assessed in a substudy of 12 HyperGEN individuals who had echocardiograms conducted two weeks apart (same echo technician, same echo reader); intraclass correlation coefficients were 0.87 for RWT and 0.90 for LVMI. Methods used to measure ARD (at end-diastole at the level of the aortic annulus and the sinuses of Valsalva) are presented in separate publications [24].

Design and statistical analyses

Linkage analysis

Genotyping for linkage analysis was performed by the Marshfield Clinic's Center for Medical Genetics—Mammalian Genotyping Service (http://research.marshfieldclinic.org/genetics/) in Marshfield, WI. A high-throughput scanning fluorescence detector system was used to genotype short tandem repeat polymorphisms. Three hundred eighty-seven polymorphic markers spaced an average of 9.7 cM apart were used for this analysis. The average heterozygosity of the markers was 0.76. Marker consistency with Mendelian expectations between siblings was tested using ASPEX, a likelihood-based method. Only confirmed siblings with markers consistent with Mendelian inheritance were used in the linkage analysis.

Race-specific genome-wide linkage analyses were conducted using standardized residual values of the phenotypes calculated using race- and sex-specific linear regression models as follows. LV mass index (LVMI, i.e., LV mass normalized by height (m)$^{2.7}$) [25], RWT and ARD were adjusted for age, age^2, and field center. Subjects whose phenotype residuals were ≥ 4 standard deviations from race- and sex-specific means (n = 17) were set to missing for

the corresponding phenotype. Final residual phenotypes were approximately normally distributed (both skewness and kurtosis < 1.0). We used multipoint variance components linkage analysis as implemented in Genehunter (version 2.1). Race-specific allele frequencies for the genetic markers were estimated based on the marker allele frequencies of randomly selected, unrelated subjects (232 of blacks and 214 whites). Linkage findings for ARD that were generated using different adjustment models and the complete HyperGEN population has been reported previously [24,26]; we have repeated these analyses here so our linkage methods would be consistent for the three phenotypes selected (i.e., the phenotypes with highest measurement reproducibility) *a priori* for the follow-up association analysis.

Candidate gene and case and control selection for association analyses

We visually identified genomic regions with generally high LOD scores (LOD ≥ 1.2, but with consideration given to clusters of smaller peaks and concordance between races) for all 3 phenotypes for both racial groups. We compared these candidate regions with known hypertrophy QTLs in the rat. Only the region on chromosome 4 showed evidence for linkage to heart weight in the rat. We then evaluated genes with known expression in human cardiac tissue from chromosome 4 and selected 7 candidate genes for our association studies (Figure 2). To reduce genotyping costs, a nested case-control approach was used instead of genotyping the full sample. Three nested case-control study groups (1 each for LVMI, RWT, and ARD) were selected as follows: For each phenotype, we identified the locus with the highest LOD score and then selected families with family-specific LOD scores ≥ 0.05 for that marker. After adjusting for age, age^2, sex, and field center, phenotype scores were sorted. *Cases* were chosen from the group of individuals with phenotype scores at or above the 67th percentile (whites: n = 134 ARD, n = 131 RWT, n = 119 LVMI; blacks: n = 229 ARD, n = 241 RWT, n = 217 LVMI); to insure cases were unrelated, only the individual with the highest phenotype score in each family was selected. *Controls* were chosen in an analogous manner, drawing from the group with phenotype scores at or below the 33rd percentile (whites: n = 86 ARD, n = 59 RWT, n = 64 LVMI; blacks: n = 122 ARD, n = 124 RWT, n = 111 LVMI) and who had a family-specific LOD score < 0; to insure controls were unrelated, only the individual with the lowest phenotype score in each family was selected. In these 3 case-control groups combined there was considerable variation in quantitative measures of LV structural phenotypes.

SNP selection and SNP genotyping

We confirmed SNPs published in public databases by resequencing each of our candidate genes in a subset of 48 unrelated individuals (12 randomly chosen LVH cases and 12 randomly chosen LVH controls in each racial stratum) and selected haplotype-tagging SNPs (htSNPs) specifically for each racial group. For genes < 10 kb we resequenced the entire gene, subsequently allowing us to select htSNPs that fully represented the haplotype structure of the gene for *each* racial group. For genes > 10 kb we aimed to identify up to 10 SNPs, giving priority to sequencing the 5′ and 3′ ends, coding regions, and SNPs in proximity to splice sites. Subsequently, we calculated linkage disequilibrium between the identified SNPs. We used the ldSelect algorithm [27] to determine haplotypes and selected representative htSNPs covering all identified haplotypes with a frequency > 10%. Genotyping for association analysis was done with a quantitative polymerase chain reaction method based on the TaqMan technology from Applied Biosystems (Foster City, CA). Samples were amplified with ABI9700 PCR thermocycler (Applied Biosystems), and fluorescence results were determined by using ABI7700 sequence detector (Applied Biosystems). Duplicate samples as well as water controls were genotyped in each plate. Most SNPs (~75%) had heterozygosity > 0.30, corresponding roughly to a minor allele

frequency > 0.2. All SNPs were in Hardy-Weinberg equilibrium. Over 95% of samples were successfully typed for most SNPs.

Association analysis and correction for multiple testing

We tested for association separately for black and white participants. We implemented a permutation-based procedure to test the gene-level hypothesis while correcting for multiple comparisons: Is any SNP in this gene associated with this trait? For each gene and trait combination, 2000 permuted data sets were generated where the trait status was randomly shuffled (since under the null hypothesis the trait and genotype are independent). For each permuted data set, including the original data set, a χ^2-statistic was computed for each SNP and the maximum χ^2 statistic among all SNPs was saved. The maximum χ^2 statistic from the original data set was then ranked against those from the permuted data sets and a permutation-based p-value was then simply calculated as 1 minus the percentile of the sample maximum χ^2 statistic.

Results

Linkage analysis

A summary of baseline anthropometric and phenotypic data is presented in Table 1. Clinical and genotype data for linkage analyses were collected from 455 whites in 187 families and 430 blacks in 195 families. The family structure is shown in Table 2. Table 3 shows all linkage peaks with LOD score ≥ 1.2. In whites, the highest linkage peaks were on chromosomes 1 (157 cM, for ARD, LOD 2.4), 9 (111 cM, for RWT, LOD 1.8), and 21 (3 cM, for LVMI, LOD 1.9). In blacks, the highest peaks were on chromosomes 1 (217 cm, for LVMI, LOD 2.0), 5 (85 cM, for ARD, LOD 2.0), and 14 (102 cM, for LVMI, LOD 2.5). We also observed a broad cluster of linkage peaks from the three phenotypes on chromosome 4 from about 110 to 180 cM in whites; although lower in magnitude, blacks also showed some linkage in this region. See Figure 1. As stated in the methods section, we prioritized the association genotyping to evaluate a genomic region showing some evidence for linkage in both ethnic groups.

Association analysis

Based on linkage results and assessment with homology with quantitative trait loci from animal models, we prioritized chromosome 4 for association testing (Figure 1). Endothelin-A receptor (EDNRA) was the only gene that did not demonstrate significant ($p < 0.05$) associations among the 7 genes studied. Among blacks, SNP-phenotype associations were significant ($p < 0.005$) in the IL15, NPY2R, and NPY5R genes. At a lower threshold of significance ($p < 0.05$) all genes (except EDNRA) showed significant association in blacks, and the effect sizes were particularly large for SNPs in the NPY2R, NPY5R, SFRP, and CPE genes. For example, the odds ratio for the variant allele for LVMI cases versus controls was 12.41 (95% confidence interval: 1.63, 94.49), 3.71 (1.61, 8.58), 2.33 (1.27, 4.30), and 2.03 (1.21, 3.41) for NPY2R rs1047214, NPY5R rs11100494, SFRP rs4076441, and CPE rs3587014, respectively. In whites, NPY2R, NPY5R, and SFRP2 showed at least 1 SNP significant at $p < 0.05$ (Figure 3), although the magnitude of the associations was consistently lower in whites than African Americans. Table 4 shows odds ratios for all SNPs in the study with at least one significant association.

Discussion

Linkage analysis

To the best of our knowledge, HyperGEN is the first study to perform genome-wide microsatellite linkage analyses for LV mass and related echocardiographic phenotypes in

large samples of black and white hypertensive sibships. Mayosi et al.'s [28] recent work reported genome-wide linkage findings for electrocardiographic and echocardiographic LV hypertrophy in 868 white individuals from the United Kingdom in pedigrees with hypertensive probands; other studies have reported linkage within candidate regions or sought to detect linkage with cardiac function phenotypes, such as stroke volume or cardiac output, but not LV mass or other structural echocardiographic traits. None of the regions of interest in these studies overlap our linkage findings [12,28,29]. The lack of replication of linkage findings is not surprising since more than half our study sample was composed of blacks while the other studies have focused on families of European descent, our phenotypes differ (quantitative measures of LVMI, RWT, and ARD rather than functional echocardiographic measures or qualitatively defined LVH), and our ascertainment differs (all hypertensive sibships). While we did not find any statistically significant evidence for linkage, we did find that the three traits – each selected because of its excellent measurement precision – showed some evidence for linkage. We also found overlap of linkage evidence for all three traits on chromosome 4 and suggestive evidence of linkage for 2 of the 3 traits in both ethnic groups. Additionally, we found homology for the region on chromosome 4 with quantitative trait loci for heart weight in the rat, and similarly, evidence that genes from this chromosomal region on chromosome 4 were associated with LV mass or blood pressure in rodents. Therefore, chromosome 4 was selected for follow-up.

Association analysis

We conducted bioinformatic searches of genes involved in LV hypertrophy in humans, rat, and/or mouse, and cross-referenced this list with the genes from the chromosome 4 region. Based on this multifaceted approach, we selected from chromosome 4 a cluster of neuropeptide Y receptor genes (NPY1R, NPY2R, and NPY5R), carboxypeptidase (CPE), endothelin-A receptor (ENDRA), secreted frizzled protein 2 (SFRP2), and interleukin 15 (IL15). We observed significant ($P<0.05$) associations with all candidate genes except EDNRA in at least 1 racial group for at least 1 of the 3 structural phenotypes, pointing to the success of our approach.

Sfrps are modulators of Wnt signaling, and the Wnt/beta-catenin pathway has been shown to be attenuated by enhanced expression of Sfrp3 and Sfrp4 (but not Sfrp2) and this may modulate myocyte apotosis in overload-induced heart failure [30]. Recently, Kobayshi et al. have demonstrated that post-MI SFRP2-null mice exhibited markedly reduced fibrosis through a signaling pathway separate from Wnt/beta-catenin [31]. Thus, SFRP2's roll in in LVH is plausible but uncertain. Carboxypeptidase E is an enzyme that cleaves the C-terminal dibasic residues arginine and lysine in prohormone precursors of NPY. Neuropeptide Y is the most abundant peptide in the heart, although its functional relevance in the myocardium remains poorly described [32]. Its cardiac action may involve a positive inotropic effect which increases Ca2+ release after activation of the NPY1 receptor [32]. A recent study suggests that the NPY1R and CPE genes may work together in determining the LV response to injury, and ischemia is associated with LV hypertrophy: Ischemic preconditioning was evaluated with respect to LV expression profiling in male Wistar rats post-injury, and the study reported that both NPY1R and carboxypeptidase (A1 in this model system) showed differential changes in the ventricle after ischemia. Among several transcripts, NPY1R was downregulated, while carboxyperptidase A1 was upregulated in the ventricle in the context of induced injury [33]. Although no reports yet exist of a potential co-regulation of these genes in humans with respect to the LV, the observation that these genes were found in this model of ischemia suggests that they may also play a role in human LV hypertrophy. We tested the statistical interaction between each of the NPY receptor genes and CPE, and found significant interactions between NPY2R and carboxypeptidase E for both LV mass and RWT (data not shown), suggesting that within the HyperGEN data,

these genes jointly play a role in the LV structural phenotype. Moreover, these genes have both been identified as important in rodent models of obesity [34,35]. Interestingly, 4 of the 7 genes investigated have been implicated in obesity (NPY1R [36]; NPY2R, including SNPs RS1047214 [37-39] and RS10461257 [38]; NPY5R [36]; and CPE [40]), suggesting that genes are pleiotropic in terms of their contribution to body size and heart size. Table 5 summarizes candidate gene associations with the 3 phenotypes and describes known gene and protein functions.

Strengths and limitations

The HyperGEN: Genetics of Left Ventricular Hypertrophy Study represents one of the largest family studies of echocardiography in the context of hypertension in 2 different racial groups. Additional strengths include the comprehensive approach used to select a region for follow-up and genes within that region, the race-specific selection of haplotype-tagging SNPs and within-race analysis to reduce the potential for population stratification, and the use of appropriate statistical methods to correct for multiple testing. This study also examined three quantitative phenotypes that capture correlated though different domains that may be influenced by different genes. We adjusted findings for covariates, including BMI, and our significant SNPs did not change with adjustment. Limitations include the absence of haplotype analysis; however, given that we resequenced genes and specifically genotyped only haplotype-tagging SNPs, we should have captured relevant haplotypes with our single-SNP approach. Nearly all hypertensive siblings were treated with antihypertensive medications; therefore, the echocardiographic traits examined may underestimate the true level of hypertrophy and/or have altered the measurement of phenotypes. We also acknowledge that different pathways and genes may influence cardiac structure in normotensive versus hypertensive individuals; therefore, our findings should not be generalized beyond hypertensive populations. We have not replicated our findings in a separate population, but we are currently genotyping all HyperGEN subjects with the Affymetrix 6.0 GeneChip and will evaluate markers in close proximity to these genes in the full cohort. Replication in an independent population is essential to judge the validity of these findings.

Conclusions

The HyperGEN Study has identified a genomic region likely to harbor susceptibility loci for LV structural or functional phenotypes, and has identified associations with SNPs within NPY1R, NPY2R, NPY5R, CPE, IL15, and SFRP2 for ARD, RWT, and/or LVMI in blacks and/or whites.

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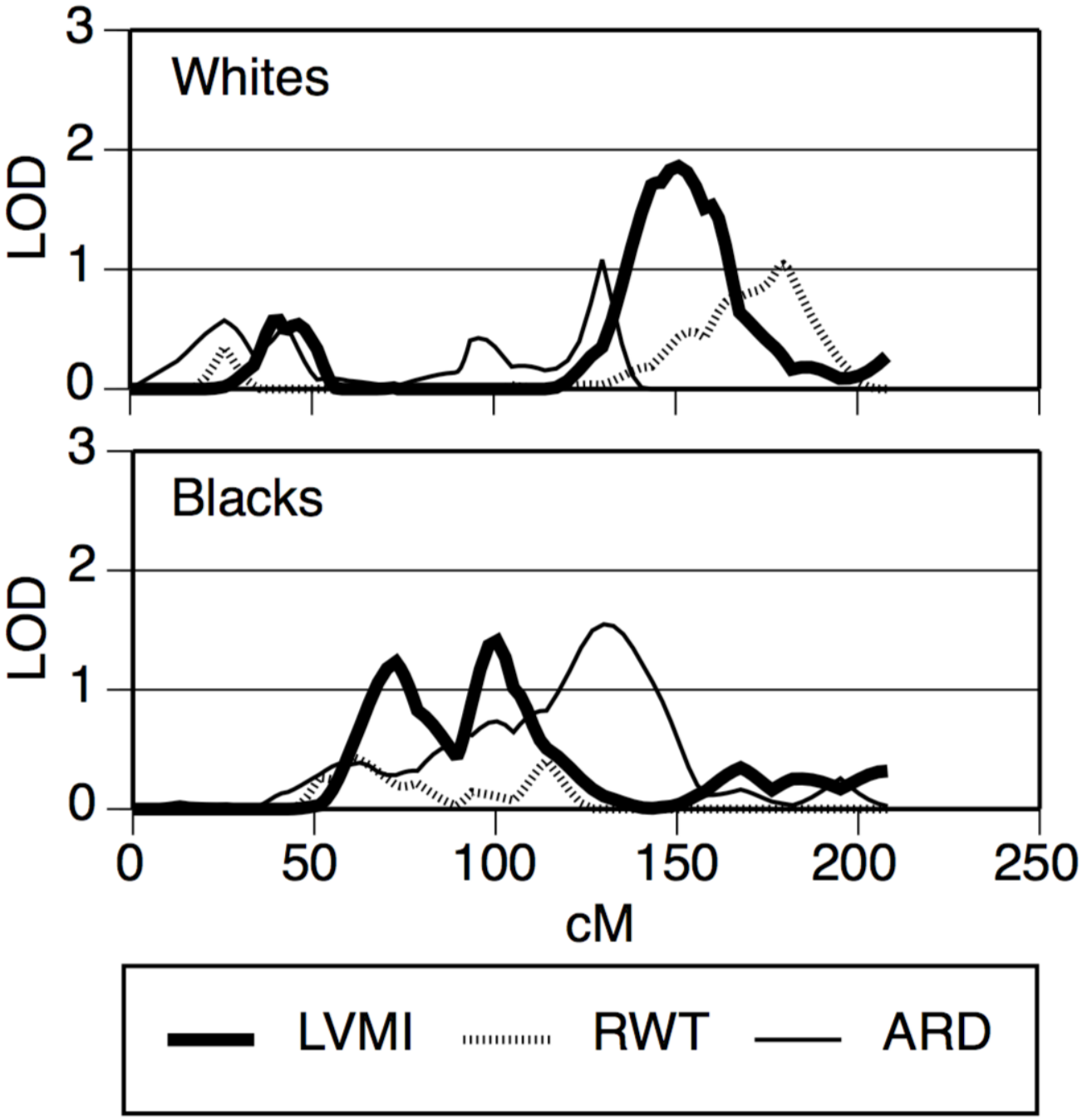

Figure 1.
Chromosome 4 linkage findings. Linkage findings used, in part, to select candidate genes for association analyses.

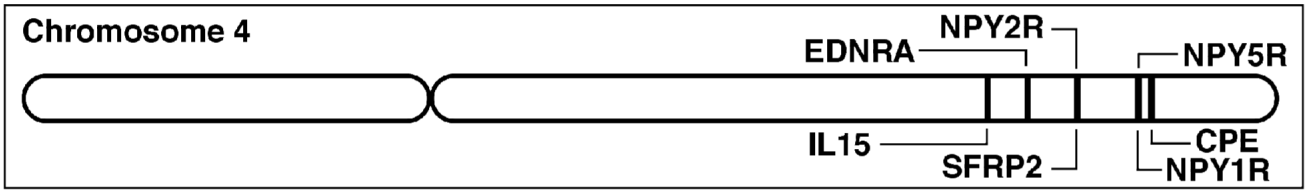

Figure 2.
Candidate genes. Approximate relative positions of chromosome 4 candidate genes.

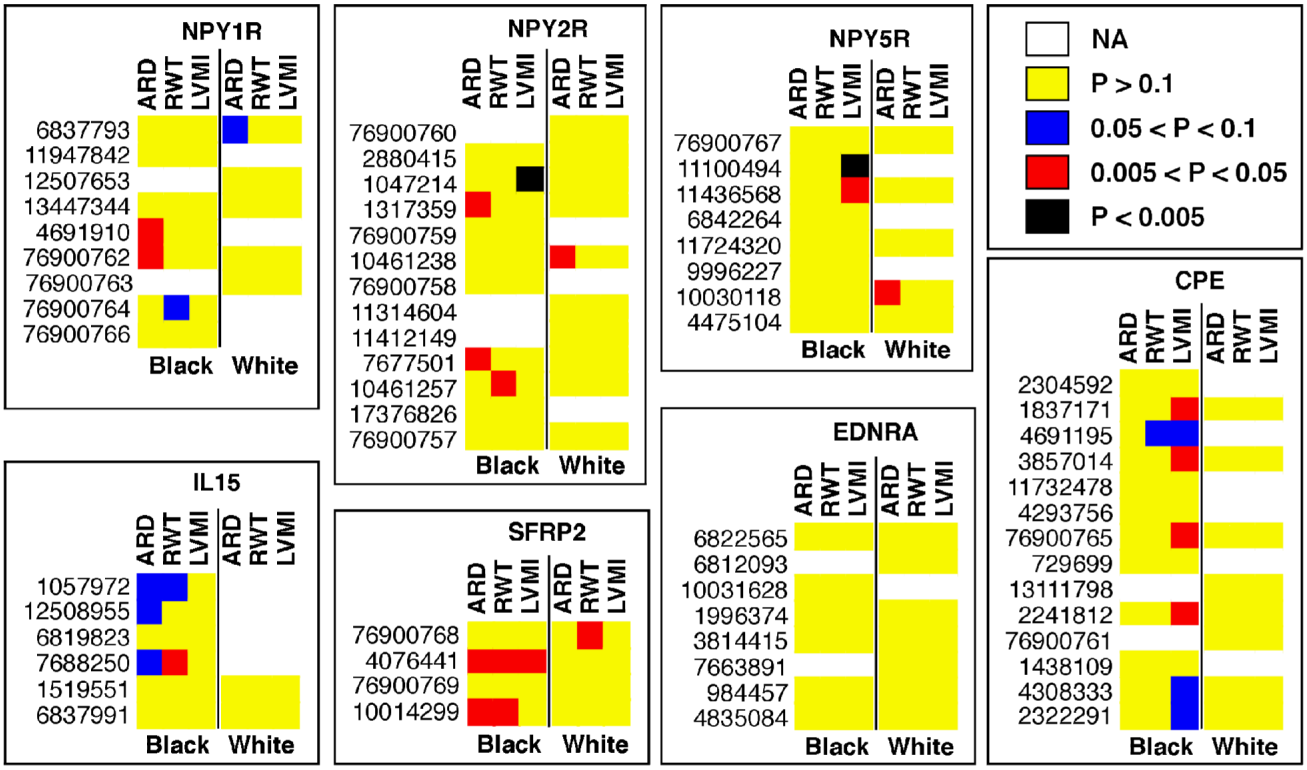

Figure 3.
Single-SNP associations. p-values for single SNP-phenotype associations.

Table 1

Descriptive statistics for those included in the linkage analysis: mean ± SD or percentage (%)

	Black (n = 430)	White (n = 455)
Age, years	52.0 ± 10.0	60.1 ± 9.0
Females, %	73.0	54.7
Weight, kg	91.3 ± 22.3	87.1 ± 19.0
Height, cm	166.3 ± 8.4	168.3 ± 9.1
BMI, kg/m^2	33.0 ± 7.8	30.7 ± 5.9
SBP, mm Hg	132.9 ± 22.3	130.5 ± 19.6
DBP, mm Hg	75.0 ± 11.3	71.3 ± 10.9
Ejection fraction, %	61.8 ± 9.4	62.2 ± 9.1
No. hypertensive medications	1.36 ± 0.87	1.39 ± 0.75
Hypertensive medication agent		
Any antihypertensive, %	87.9	92.3
ACE inhibitors, %	30.6	38.1
Calcium channel blockers, %	44.6	27.8
Beta-blockers, %	14.8	27.6
Diuretics, %	57.6	41.5
Other, %	2.33	5.49
Diabetes, %	23.3	19.3
Phenotype means ± SD (N)		
RWT	0.36 ± 0.06 (413)	0.35 ± 0.05 (432)
LVMI, $g/m^{2.7}$	45.2 ± 12.2 (412)	42.1 ± 9.6 (429)
ARD, cm	3.34 ± 0.36 (426)	3.51 ± 0.40 (451)

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACE, angiotensin converting enzyme; RWT, relative wall thickness; LVMI, Left ventricular mass indexed to height; ARD, aortic root diameter.

Table 2

Distribution of families included in the linkage analysis. Only family members who had both echo measurements and genetic markers were included

	Black	White
Families	195	187
Family size range	2-4	2-7
Individuals	430	455
Sib pairs	226	358
Half-sib pairs	44	10
Parent-child pairs	9	7
Avuncular pairs	2	13
Total relative pairs	281	388

Table 3

Linkage peaks with LOD score ≥ 1.2

Chr	cM	Pheno	LOD
Black			
1	108	RWT	1.4
1	217	LVMI	2.0
4	73	LVMI	1.2
4	100	LVMI	1.4
4	130	ARD	1.5
5	41	ARD	1.2
5	85	ARD	2.0
6	80	LVMI	1.2
10	49	ARD	1.3
12	56	ARD	1.3
14	31	LVMI	1.3
14	102	LVMI	2.5
17	126	ARD	1.8
21	40	RWT	1.3
White			
1	157	ARD	2.4
4	151	LVMI	1.9
5	41	RWT	1.3
7	67	LVMI	1.6
8	140	LVMI	1.4
9	111	RWT	1.8
12	95	LVMI	1.2
21	3	LVMI	1.9

Pheno, phenotype; Chr, chromosome; cM, position in centimorgans; LOD, logarithm of the odds score; B, black; W, white; RWT, relative wall thickness; LVMI, left ventricular mass indexed to height; ARD, aortic root diameter.

Table 4

Odds ratios (95% confidence intervals) for cases and controls for the presence of the variant SNP allele. Data shown only for those SNPs with at least one significant (P < 0.05) association whose 95% confidence interval did not include 0

	Black			White		
RS#	**ARD**	**RWT**	**LVMI**	**ARD**	**RWT**	**LVMI**
NPY1R						
76900762	**1.62 (1.03, 2.54)**	0.95 (0.61, 1.48)	1.15 (0.71, 1.84)	0.80 (0.45, 1.43)	0.97 (0.5, 1.86)	1.05 (0.55, 2)
NPY2R						
10461238	1.01 (0.59,1.73)	0.81 (0.48, 1.39)	0.64 (0.35, 1.15)	**1.90 (1.09, 3.34)**	1.11 (0.58, 2.12)	0.79 (0.42, 1.50)
1047214	1.34 (0.47, 3.81)	0.75 (0.31, 1.84)	**12.41 (1.63, 94.49)**	0.87 (0.47, 1.59)	1.02 (0.52, 2.00)	1.04 (0.53, 2.05)
NPY5R						
10030118	0.87 (0.56, 1.36)	0.95 (0.61, 1.47)	1.04 (0.65, 1.65)	**2.45 (1.05, 5.72)**	1.23 (0.53, 2.85)	0.76 (0.38, 1.52)
11436568	0.83 (0.52, 1.32)	0.94 (0.59, 1.48)	**1.61 (1, 2.58)**	0.89 (0.51, 1.55)	0.87 (0.47, 1.61)	1.2 (0.65, 2.22)
11100494	1.11 (0.6, 2.05)	0.82 (0.45, 1.5)	**3.71 (1.61, 8.58)**	NA	NA	NA
SFRP2						
10014299	**2.31 (1.16, 4.62)**	**2.26 (1.13, 4.51)**	1.34 (0.68, 2.64)	1.05 (0.58, 1.89)	1.07 (0.57, 2.00)	1.52 (0.79, 2.94)
4076441	**1.99 (1.15, 3.46)**	**1.90 (1.10, 3.28)**	**2.33 (1.27, 4.30)**	0.80 (0.44, 1.46)	0.84 (0.44, 1.61)	0.65 (0.34, 1.23)
76900768	1.09 (0.70, 1.72)	1.32 (0.84, 2.06)	1.03 (0.65, 1.65)	0.95 (0.49, 1.86)	**2.32 (1.15, 4.68)**	0.96 (0.47, 1.97)
CPE						
2241812	0.77 (0.49, 1.20)	1.13 (0.73, 1.76)	**1.81 (1.12, 2.92)**	1.31 (0.71, 2.41)	0.90 (0.44, 1.84)	1.17 (0.57, 2.38)
76900765	0.88 (0.57, 1.37)	1.01 (0.65, 1.57)	**1.77 (1.10, 2.85)**	1.06 (0.57, 1.98)	0.95 (0.47, 1.93)	0.93 (0.45, 1.90)
3587014	0.79 (0.50, 1.24)	1.08 (0.68, 1.72)	**2.03 (1.21, 3.41)**	1.04 (0.56, 1.92)	1.5 (0.76, 2.96)	1.01 (0.52, 1.97)
1837171	0.97 (0.62,1.52)	1.02 (0.65, 1.58)	**1.60 (1.00, 2.56)**	0.69 (0.40, 1.20)	1.06 (0.57, 1.98)	1.09 (0.59, 2.00)

NA, not available; RWT, relative wall thickness; LVMI, left ventricular mass indexed to height; ARD, aortic root diameter. Individual SNP associations that are statistically significant (P < 0.05) are in **bold text**.

Table 5

Summary of the Positional Candidate Gene Functions and Associations within HyperGEN

Gene (tests†)	Number of associations p < 0.05* B	W	Documented protein functions and phenotype associations	Ref
NPY1R (27)	2	0	Npy acts as a potent vasoconstrictor via the Y1 receptor. Npyr1 has also been implicated in Npy-mediated vascular smooth muscle growth.	[41,42]
NPY2R (39)	4	1	Tsujita et al. found an LVM QTL containing NPY2R. In a rat model, an Npy2r antagonist significantly reduced BP and development of LVH independently of BP change. Npy2r (along with Npy5r) has been implicated in angiogenesis.	[41-43]
NPY5R (24)	2	1	The receptor is responsible for mediating NPY-induced food intake in rats and likely plays a key role in energy homeostasis. Npy5r (along with Npy2r) has been implicated in angiogenesis.	[41,42,44-46]
SFRP2 (12)	5	1	The Wnt/Frizzled pathway is known to play a role in cardiac hypertrophy development. Schumann et al. reported that Sfrp3 and Sfrp4—but not Sfrp2—attenuate Wnt/beta-catenin pathway in failing human myocardium. Kobayashi et al. showed post-MI SFRP2-null mice exhibited markedly reduced fibrosis. Therefore, SFRP2's role in LVH is plausible, but unknown.	[30,31]
CPE (42)	4	0	Cpe has a well documented role in the production of peptides such as insulin.	[47]
IL15 (18)	1	0	Il15 is a cytokine that regulates T and natural killer cells-15 concentrations in essential hypertension patients with organ damage (including LVH) is higher than that in those with no or mild organ damage. IL-15 concentration is correlated with cardiovascular disease independently of blood pressure.	[48]
EDNRA (24)	0	0	Receptor for Edn1, which inhibits active Na-K transport in renal tubules and other tissues. Transduces the vasoconstrictive properties of endothelin-1. EDNRA mediates Edn1's effect of increasing the diastolic distensibility of acutely loaded human myocardium. Likely plays a role in baroreflex sensitivity and the development of essential hypertension. Associated with idiopathic dilated cardiomyopathy and aortic pressure in patients with dilated cardiomyopathy.	[49-53]

* p < 0.05 for associations with aortic root diamter, relative wall thinkness, and left ventricular mass index

† Tota l number of SNP-phenotype associations tested for the gene in each race.