Linkage of Left Ventricular Contractility to Chromosome 11 in Humans

The HyperGEN Study

Donna K. Arnett, Richard B. Devereux, Dalane Kitzman, Al Oberman, Paul Hopkins, Larry Atwood, Andrew Dewan, D.C. Rao

Abstract—Impaired left ventricular (LV) contractility is a major cause of cardiovascular death, especially congestive heart failure. The identification of susceptibility genes that contribute to impaired LV contractility may uncover mechanisms underlying LV contractile impairment and the development of congestive heart failure. The Hypertension Genetic Epidemiology Network (HyperGEN) collected echocardiographic measurements of myocardial contractility in a large biethnic sample of hypertensive siblings (390 blacks and 398 whites in 179 and 165 sibships, respectively). All participants expressed hypertension before age 60 years, and the mean age of siblings was 52 years in blacks and 61 years in whites. We adjusted myocardial contractility for gender, age, and age², and we calculated standardized residuals separately for men and women in both ethnic groups. We conducted multipoint variance components linkage analysis using GENEHUNTER2 and 387 anonymous markers (CHCL8 marker set). We found evidence for significant linkage to a microsatellite marker, D11S1993 (lod, 3.93 in blacks), '54 cM from the tip of the short arm of chromosome 11, that accounted for 72% of the phenotypic variation in LV contractility. A chromosome 22 locus showed suggestive evidence for linkage (lod, 2.83 in whites and 1.15 in blacks). The chromosome 11 peak coincides with the region containing myosin-binding protein C. Mutations in this gene are linked to familial hypertrophic cardiomyopathy. Our results show strong evidence for linkage of a region of chromosome 11 with LV contractility in blacks and suggest that an important gene for impaired LV contractility is harbored in this region. (Hypertension. 2001;38:767-772.)

Key Words: genes ▪ gene expression ▪ myocardium ▪ hypertension, genetic ▪ race

Impaired left ventricular (LV) contractility profoundly affects morbidity and mortality rates from cardiovascular diseases, including myocardial infarction, congestive heart failure, and stroke.1 Familial forms of severe LV hypertrophy that result in impaired LV contractility are reported, and mutations in several genes that contribute to these conditions have been identified.2-6 However, little is known regarding the genetic underpinnings of more common forms of impaired LV contractility that likely account for most of the disease burden in humans.

Noninvasive imaging methods, such as echocardiography, have greatly expanded the ability to evaluate cardiac structural and functional characteristics and have enhanced understanding of the natural history of impaired LV contractility. To evaluate the genetic contributions to impaired LV contractility, the Hypertension Genetic Epidemiology Network (HyperGEN) study,7 which was designed to identify genes that contribute to hypertension, collected echocardiographic measures of LV contractility in hypertensive siblings. Echocardiographically determined stress-corrected midwall shortening (MWS), measured at the level of the minor axis,8-11 is reproducible,12 and when applied to a study of hypertensive patients,13 it can be used to predict an adverse prognosis independent of LV mass, age, and blood pressure.1 The genetic basis for genetic contributions to interindividual variation of this measure is not reported. The goal of the present study was to identify chromosomal regions linked to interindividual variation in stress-corrected MWS, an index of LV contractility.

Methods

Study Population
Subjects included blacks and whites from 344 sibships that ranged in size from 2 to 7 individuals (Table 1) and were participating in HyperGEN. HyperGEN is 1 of 4 networks in the National Heart, Lung, and Blood Institute Family Blood Pressure Program, a study designed to identify genetic contributions to hypertension.7 Probandswere identified on the basis of an onset of hypertension by age 60 years and the presence of ≥1 additional hypertensive sibling who

Received February 13, 2000; first decision March 8, 2000; accepted March 8, 2001.
From the Division of Epidemiology, University of Minnesota (D.K.A., L.A., A.D.), Minneapolis; Cornell University Medical College (R.B.D.), New York, NY; Wake Forest University School of Medicine (D.K.), Winston-Salem, NC; Division of Preventive Medicine, University of Alabama (A.O.), Birmingham; University of Utah (P.H.), Salt Lake City; and Division of Biostatistics, Washington University School of Medicine (D.C.R.), St Louis, Mo.
Correspondence to Donna K. Arnett, PhD, Division of Epidemiology, University of Minnesota, 1300 S Second St, Suite 300, Minneapolis, MN 55454.
E-mail arnett@epi.umn.edu
© 2001 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org

767
was willing to participate. Hypertension was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg on ≥2 different evaluations or self-reported treatment for hypertension. Volunteers with type 1 diabetes mellitus or renal failure were excluded to remove potential secondary causes of hypertension. Subjects were recruited from existing cohort studies or from the community-at-large. Four of the 5 field centers in HyperGEN (Birmingham, Ala; Forsyth County, NC; Minneapolis, Minn; and Salt Lake City, Utah) participated in the ancillary echocardiographic study, which generated data for this report. At Birmingham, blacks were recruited exclusively; at Winston-Salem, ≈50% blacks and ≈50% whites were recruited; and at Minneapolis and Salt Lake City, whites were recruited exclusively.

### Phenotyping

Echocardiograms were performed using a standardized protocol with phased-array echocardiographs with M-mode, 2D and pulsed-, continuous-wave, and color flow Doppler capabilities. Examinations were performed with tables with cut-outs to facilitate apical imaging. The head of the examining table was elevated 30°, and a partial decubitus position was maintained. From the parasternal acoustic window, ≥10 consecutive beats of 2D and M-mode recordings of the LV internal diameter and wall thicknesses were made at or just below the tips of the anterior mitral leaflets in both long- and short-axis views, long-axis views of the mitral valve, and color flow recordings to search for mitral and aortic regurgitation. The apical window was used to record ≥10 cycles of 2- and 4-chamber images and color Doppler recordings to assess LV wall motion and to identify mitral and aortic regurgitation. All elements of the protocol were recorded on videotape. Principal sonographers received centralized training at the Reading Center in New York.

Correct orientation of planes for imaging and Doppler recordings was verified according to standard procedures. LV internal dimensions and wall thicknesses were measured at end diastole and end systole according to American Society of Echocardiography recommendations. When optimal orientation of the LV M-mode beam could not be obtained, correctly oriented linear dimension measurements were made using 2D imaging by the leading-edge convention. Wall motion was assessed using the parasternal long- and short-axis and apical views. Echocardiograms were read by technical readers and over-read by physician readers. All readers were blinded to subjects' clinical data.

End-diastolic LV dimensions were used for measurements, and end-systolic LV volumes were calculated according to the method of Teichholz et al. Systolic function was assessed by MWS, a measure of myocardial contractile efficiency, corrected for end-systolic stress, measured at the level of the LV minor axis. Standardized anthropomorphic measurements included body mass index, body surface area, and percent body fat by bioelectric impedance to calculate fat-free body mass and adipose mass as the difference between body weight and fat-free mass. Prevalent coronary heart disease was defined by self-report.

### Genotyping

Genotyping was carried out by the NHLBI Mammalian Genotyping Service. The CHLC Screening Set 8 was used, which includes 387 microsatellite markers spaced at approximately equal intervals every 9 cM throughout the genome. The average marker heterozygosity was 0.76. Analyses and assignment of the marker alleles were made with computerized algorithms. Relationship status among the purportedly full sibs was tested using ASPEX, a likelihood-based method. Only confirmed full sibs were used in the linkage analysis, and the numbers in Table 1 reflect such exclusions.

### TABLE 1. Number of Siblings per Sibship in Blacks and Whites

<table>
<thead>
<tr>
<th>No. of Siblings</th>
<th>Blacks</th>
<th>Whites</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>151</td>
<td>113</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>165</td>
</tr>
</tbody>
</table>

### TABLE 2. Demographic, Anthropometric, and Echocardiographic Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Blacks</th>
<th>Whites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n=102)</td>
<td>Women (n=288)</td>
<td>Men (n=180)</td>
</tr>
<tr>
<td>Age, y</td>
<td>51.6 (9.0)</td>
<td>51.5 (10.2)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>94.0 (20.6)</td>
<td>90.4 (22.9)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.76 (0.06)</td>
<td>1.63 (0.06)</td>
</tr>
<tr>
<td>Prevalent CVD, %</td>
<td>13.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>133.4 (21.8)</td>
<td>132.7 (22.5)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>79.2 (11.9)</td>
<td>73.6 (10.9)</td>
</tr>
<tr>
<td>Age of HBP onset, y</td>
<td>37.8 (10.6)</td>
<td>36.6 (10.5)</td>
</tr>
<tr>
<td>Stress-corrected MWS</td>
<td>100.9 (14.8)</td>
<td>102.9 (13.1)</td>
</tr>
</tbody>
</table>

**CVD** indicates cardiovascular disease; **BP**, blood pressure; **HBP**, high blood pressure; **MWS**, midwall shortening; **PWT**, posterior wall thickness; **IVST**, intraventricular septal thickness; and **LVID**, left ventricular internal diameter.

Values are mean (STD).
**TABLE 3.** Chromosome, Marker Name, Location, Multipoint Lod Scores, Phenotypic Variance, and QTL Variance for Blacks and Whites

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Marker</th>
<th>Location, cm</th>
<th>Blacks Lod</th>
<th>Vp</th>
<th>VQTL</th>
<th>Whites Lod</th>
<th>Vp</th>
<th>VQTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>D11S1993</td>
<td>54.09</td>
<td>3.97</td>
<td>182.3</td>
<td>130.7</td>
<td>0.00</td>
<td>166.2</td>
<td>0.00</td>
</tr>
<tr>
<td>12</td>
<td>D12S398</td>
<td>58.63</td>
<td>0.00</td>
<td>182.3</td>
<td>0.00</td>
<td>1.97</td>
<td>166.2</td>
<td>78.1</td>
</tr>
<tr>
<td>22</td>
<td>D22S420</td>
<td>17.94</td>
<td>1.15</td>
<td>182.3</td>
<td>81.5</td>
<td>2.83</td>
<td>166.2</td>
<td>116.8</td>
</tr>
</tbody>
</table>

Vp indicates phenotypic variance; and VQTL, QTL variance.

**Linkage Analysis**

Before linkage, we adjusted stress-corrected MWS for age, age², gender, and ethnicity. We output the residuals and standardized them to a mean of zero, and we used these residuals in the linkage analysis. For linkage, we used a multipoint variance components model, GENEHUNTER, which estimates the amount of variance in a quantitative trait attributable to a quantitative trait locus (QTL) at that position. Maximum likelihood values for the mean trait value, additive and dominant variance components for the QTL, additive and dominant components for other unlinked loci, and an environmental variance were calculated. To test the hypothesis that the genetic variance due to the QTL equaled zero, the likelihood when the variance components were estimated was compared with the likelihood when the variance components were constrained to zero. The difference between the 2 log likelihoods mental variance were calculated. To test the hypothesis that the additive and dominant variance for the QTL, additive position. Maximum likelihood values for the mean trait value, quantitative trait attributed to a quantitative trait locus (QTL) at that position. Maximum likelihood values for the mean trait value, additive and dominant variance components for the QTL, additive and dominant components for other unlinked loci, and an environmental variance were calculated. To test the hypothesis that the genetic variance due to the QTL equaled zero, the likelihood when the variance components were estimated was compared with the likelihood of the model in which the variance components were constrained to zero. The difference between the 2 log likelihoods yields the lod score. Models were also constructed in which the dominance variance was not calculated. The multipoint identical-by-descent distribution was calculated using an exact approach, which extracts the full probability distribution of allele sharing across a chromosome. Allele frequencies were estimated separately in blacks and whites with the random sample.

**Results**

In this sample of hypertensive siblings, the mean level MWS was 100.9% (men) to 102.9% (women) of that predicted in blacks and 101.8% (men) to 105.2% (women) of that predicted in whites, respectively (Table 2). We detected 1 chromosome with a maximum multipoint lod score of >1.75 in blacks and 2 such chromosomes in whites (Table 3). We selected 1.75 because it corresponds to, on average, 1 false positive per genome scan using 400 markers. The maximum lod score detected in the multipoint analysis was on chromosome 11 in blacks (marker D11S1993); there was no corresponding evidence for linkage in this region in whites (Figure). The marker locus accounted for 72% of the variation in stress-corrected MWS in blacks. The second largest multipoint lod score was on chromosome 22 (marker D22S420) for whites; the multipoint lod score for blacks at the same marker was lower (Figure). The locus on chromosome 22 accounted for 67% of the variation in whites and 18% of the variation in blacks. In addition, chromosome 12 (marker D12S398) showed a maximum lod score of 1.97 (Table 3), which met the Lander and Kruglyak criterion for suggestive linkage.

We eliminated 22 individuals with moderate to severe wall motion abnormalities and repeated the linkage analyses on chromosomes 11, 12, and 22. The peak lod score on chromosome 11 was modestly attenuated (2.6 in blacks), but there was little change in the findings for the other 2 chromosomes (data not presented).

**Discussion**

Our data indicate strong evidence for linkage of a region of chromosome 11 with LV contractility in blacks and suggest that this region could contain an important gene for impaired LV contractility. That intrindividual variation in LV structure and function has a significant genetic component that has emerged from several lines of research, including studies conducted in animals and humans. Impaired LV contractility and hypertrophy are pathogenetically related, although the temporal sequence between hypertrophy and impaired contractility is debated. LV hypertrophy attenuates the capacity of the ventricle to buffer sudden changes in intracellular calcium, and calcium is important for LV contractility. Modifications in the expression of genes encoding the sarcomere, calcium transport and binding, and cell signaling systems are associated with phenotypic variation in LV mass, geometry, and systolic function. More than 100 mutations in genes that encode sarcomeric proteins have been identified that lead to monogenic forms of hypertrophy, namely, familial hypertrophic cardiomyopathy, a condition present in 1% to 2% of the adult population. We used genetic linkage to investigate whether genomic regions that contain genes contributing to monogenic disorders (eg, hypertrophic cardiomyopathy) explain quantitative variation in interindividual susceptibility to impaired LV contractility among hypertensives.

Of particular relevance to our linkage result is the cardiac myosin-binding protein (MyBP-C). MyBP-C, located in the same region in chromosome 11 (11p11.2) at which we found significant linkage, consists of 37 exons. The gene encodes a large, abundant myofibrillar protein with both structural and regulatory functions. The developmental onset of expression of MyBP-C corresponds to the appearance of cross striations, implying that MyBP-C plays a role in the alignment of thick filaments within the sarcomere. MyBP-C has several functions: it binds myosin heavy chain and titin, stimulates cardiac actomyosin ATPase, and influences myofibril tension generation and contractile velocity. In addition, MyBP-C is phosphorylated by a catecholamine-mediated pathway that dynamically regulates contraction. This pathway may serve a dominant negative mutation by adversely affecting phosphorylation of MyBP-C during states of heightened adrenergic tone. Mutations in MyBP-C account for 15% to 20% of familial hypertrophic cardiomyopathy and exhibit a later age of onset and a milder form of hypertrophic cardiomyopathy than do mutations in other sarcomeric proteins. Despite considerable knowledge of the structure and function of MyBP-C, the mechanisms by which MyBP-C muta-
tions cause hypertrophic cardiomyopathy in humans are not well defined. Neither reduced peptide levels nor mutant MyBP-C peptides have been found in cardiac tissues of affected individuals. To test the functional consequence of mutations in MyBP-C, a mouse model was created in which mice \((n=10)\) were homozygous for a MyBP-C mutation that encoded a truncated protein that phenotypically expressed dilated cardiomyopathy. Homozygous mutant mice had larger echocardiographically determined LVs and were found to have poorer LV systolic function than with wild-type mice. The mutant mice had myocyte hypertrophy, myofibrillar disarray, fibrosis, and dystrophic calcification. However, sarcomeres had near-normal banding patterns and sarcomere length, suggesting that MyBP-C has a nonessential role in forming and maintaining sarcomere ultrastructure or that the protein portion expressed by the mutant MyBP-C is adequate for this function. In contrast to the homozygous mice, heterozygous mice expressed hypertrophic cardiomyopathy, suggesting that the degree of myocardial contractility acted as a central signaling mechanism that triggered different pathways for geometric remodeling. External forces, such as hemodynamic load or extracellular matrix modeling, could

Genome scan plots for chromosomes 11 and 22. On the X axis are the marker names; on the Y axis, the lod scores for the markers used in the linkage scan.
exacerbate sarcomeric dysfunction and convert compensated hypertrophy to uncompensated failure. In our study, we observed differences in linkage results between ethnic groups. Because black participants in HyperGEN had, on average, higher blood pressures than whites, it may be that the linkage on chromosome 11 was detected because of the increased hemodynamic load associated with hypertension in blacks.

Suggestive linkage was detected on chromosome 12 (lod, 1.97, whites only) and chromosome 22 (lod, 1.15 in blacks and 2.83 in whites). The Framingham Heart Study also detected linkage of LV mass, a correlated phenotype, near the peak on chromosome 22 detected in our study.32 A search for genes in the linkage regions identified only 1 potential candidate: the \( \beta \)-adrenergic receptor kinase 2 (ADRBK2). Adrenergic receptors play a critical role in abnormalities of LV function.33 ADRBK2 is a member of the \( G \) protein–coupled receptor kinase family, which regulate \( \beta \)-adrenergic signaling. In heart disease, ADRBK mRNA, protein level, and enzymatic activity are increased, further contributing to attenuation in \( \beta \)-adrenergic signaling and, ultimately, the contractile dysfunction seen in human heart disease.

Poor LV contractile function may arise through different pathways, involving complex interactions among the environment, genes that regulate LV contractility, and genes that contribute to LV structure. The effect of a putative allele within a given locus may be expressed only in the presence of a superimposed environmental context. The linkage on chromosome 11 was observed exclusively in blacks. Blacks, who have an earlier age of onset of hypertension, a higher level of blood pressure both with and without treatment, and a greater LV mass relative to whites, may express the genetic susceptibility to poor LV contractility because of this environmental context. Indeed, these blood pressure patterns were observed in blacks in the HyperGEN sample (Table 2). Longitudinal studies that track the environmental, biologic, and genetic background with disease progression may shed important light on how these factors interact to cause impaired LV contractility. The differences in linkage results between races may also be due to differences in the prevalence of LV diseases between the ethnic groups. To account for these potential differences, 22 individuals with moderate to severe wall motion abnormalities were excluded, and linkage was reevaluated in the 3 chromosomes with suggestive or significant linkage. Although the results for chromosome 11 were somewhat attenuated in blacks, there still was evidence for suggestive linkage in the absence of those with the most seriously impaired systolic function, indicating that differences in disease prevalence is not a likely explanation of the observed results.

In conclusion, these results provide strong evidence for linkage with LV contractility (lod, 3.93) in the region of chromosome 11 containing the gene that encodes MyBP-C. A suggestive region on chromosome 22 demonstrates linkage to LV mass in another population. Corroboration of our findings is provided by other studies that involve a severe form of hypertrophy and LV contractile dysfunction: hypertrophic cardiomyopathy.2-4 Therefore, these regions on chromosome 11 and 22 may contain important genes that determine systolic function of the LV.

Acknowledgments

The HyperGEN network is funded by National Heart, Lung, and Blood Institute grant R01-HL-5673 and cooperative agreements (U10) with NHLBI: HL-54471 (UT FC), HL-54472 (MN Lab), HL-54473 (DCC), HL-54495 (AL FC), HL-54496 (MN FC), HL-54509 (NC), and HL-54515 (UT DNA Lab).

References


12. Palmieri V, Dahlof B, DeQuattro V, Sharp N, Bella JN, de Simone G, Roman MJ, Devereux RB, DeMaria A, Schiffrin EL, Tajik AJ. Recommendations for quantification of the left ventricle by...


Linkage of Left Vventricular Contractility to Chromosome 11 in Humans: The HyperGEN Study
Donna K. Arnett, Richard B. Devereux, Dalane Kitzman, Al Oberman, Paul Hopkins, Larry Atwood, Andrew Dewan and D.C. Rao

Hypertension. 2001;38:767-772
doi: 10.1161/hy1001.092650

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/38/4/767

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/