Genome-wide linkage analysis of electrocardiographic and echocardiographic left ventricular hypertrophy in families with hypertension

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Aims

To localize chromosomal regions (or quantitative trait loci) that harbour genetic variants influencing the variability of electrocardiographic (ECG) and echocardiographic left ventricular hypertrophy (LVH).

Methods and results

We evaluated genetic linkage to ECG Sokolow-Lyon voltage, ECG Cornell voltage product, ECG left ventricular (LV) mass, and to echocardiographic septal wall thickness, LV cavity size, and LV mass in 868 members of 224 white British families. A genome-wide scan was performed with microsatellite markers that covered the genome at 10-cM intervals and linkage was assessed by variance components analysis. We identified chromosomal regions suggestive of linkage for Sokolow-Lyon voltage on chromosome 10q23.1 (log10 of the odds (LOD = 2.21, P = 0.0007)), for ECG Cornell voltage product on chromosome 17p13.3 (LOD = 2.67; P = 0.0002), and for ECG LV mass on chromosome 12q14.1 (LOD = 2.19; P = 0.0007). There was a single region of possible linkage for echocardiographic LV mass on chromosome 5p14.1 (LOD = 1.6; P = 0.003).

Conclusion

Stronger genetic signals for LVH were found using electrocardiographic than echocardiographic measurements, and the genetic determinants of each of these appear to be distinct. Chromosomes 10, 12, and 17 are likely to harbour genetic loci that exert a major influence on electrocardiographic LVH.

Keywords

Electrocardiogram • Echocardiogram • Left ventricular hypertrophy • Genetics • Linkage analysis • Quantitative trait loci

Introduction

Left ventricular hypertrophy (LVH) measured by the electrocardiogram (ECG) or the echocardiogram (echo) is a major risk factor for cardiovascular morbidity and mortality.1,2 Whereas LVH is a multifactorial trait that is influenced by blood pressure (BP), body size, and gender, there is increasing evidence showing that genetic factors influence the inter-individual variation in this trait.3–11 The specific genetic loci that influence naturally occurring variation in LVH, however, remain largely unknown.12 The insertion/deletion polymorphism in intron 16 of the angiotensin-converting enzyme (ACE) gene was the first polymorphism to be implicated in LVH,13 but subsequent investigations of this association have produced conflicting results.14 Numerous other candidate gene studies, conducted in the general population and in people with hypertension, have revealed either the absence of
We have conducted a whole genome linkage analysis in families with hypertension to identify novel quantitative trait loci that influence electrocardiographic and echocardiographic LVH. We have studied families who were selected through a hypertensive proband, resulting in a high prevalence of ECG-LVH and echo-LVH of 10% and 34%, respectively. In contrast with previous studies that have analysed echocardiographic LVH, we have extended our analysis to include electrocardiographic measures of LVH, which we have previously shown to be heritable. This study thus provides the most comprehensive analysis of this question to date.

Methods

Study participants

Two hundred and forty-eight white British families, comprising 1428 individuals, were recruited in Oxfordshire between 1993 and 1997 as previously described. Briefly, families were selected through a hypertensive proband with documented systolic and diastolic BP in the top 5% of the population distribution either on multiple clinical readings or ambulatory BP monitoring. In order to be suitable for the study, families were required to consist of at least three siblings (including the proband) clinically assessable for BP at least one parent of the sibship was available to give blood for DNA analysis, and to consist of at least four assessable siblings (including the proband) if no parent was available for DNA analysis. Qualifying sibships could be either in the generation of the proband or his/her offspring, and there was no requirement for the sibship to contain additional members affected with hypertension (though this was not an exclusion criterion). Where members of the sibship were found to be hypertensive (using identical criteria to those applied in the proband), families were extended and the spouses and offspring of hypertensive proband, resulting in a high prevalence of ECG-LVH and echo-LVH. We have conducted a whole genome linkage analysis in families on whom ECG data were available.

Results

The clinical characteristics and mean values for ECG and echocardiographic measures of LVH in the 868 participants from 224 families on whom ECG data were available are presented in Table 1. The median number of individuals with ECG and echo phenotypes in each family was four, and 168 of 224 families comprised between two and six phenotyped and genotyped members. The median duration of hypertension among those meeting the study criterion for hypertension was 5.8 years (IQR 2.2–11.6

Genotyping

DNA was extracted from whole blood or buffy coat specimens by a standard protocol. The 400 microsatellite markers comprising ABI PRISM linkage marker set MD-10, which span the genome with approximately 10-cM density and mean heterozygosity 0.79, were typed by polymerase chain reaction (PCR) and allele separation on ABI 377 sequencers using publicly available protocols (www.cng.fr). A CEPH reference individual was included on all genotyping runs to ensure consistency of allele binning across runs. Mendelian inconsistencies were detected and eliminated with PedCheck.

Statistical analyses

Regression analysis was used to derive sex-specific standardized residuals for LVH variables, adjusted for major covariates with SPSS as previously described. Age, systolic BP, weight, and waist-hip ratio were significant covariates for both ECG and echocardiographic measures of LV mass; height was a significant covariate for ECG measures of LV mass; and diabetes was a significant covariate for echocardiographic LV mass. The standardized residuals from the regression conformed to a normal distribution (P > 0.05). A genome-wide linkage analysis using these residuals as continuous traits was performed by a variance components approach implemented in MERLIN. Briefly, the variance–components linkage approach involves the fitting of a linear mixed model to identity-by-descent and quantitative trait data. For each chromosome and pair of relatives, identity-by-descent probabilities are computed at a regular grid of genetic locations using multilocus genotypes. The mixed model includes parameters to simultaneously estimate the trait mean with three components of variance attributable to (1) additive effects encoded by a quantitative trait locus (QTL), (2) unlinked polygenes, and (3) random, normally distributed individual-specific effects. By convention, a LOD (log_{10} of odds ratio) score test is used to summarize the evidence for a linked QTL at each genomic location; this test compares the general model (with the maximum likelihood estimate of the QTL variance component) with a nested model (with the QTL variance component = 0).

Theoretical work based on the availability of complete genetic information (i.e. no failed genotypes in typed individuals, complete availability of parental genotypes, and a high density of genetic markers) has identified LOD score cut-offs of 2.20 for suggestive evidence of linkage and 3.63 for significant evidence of linkage in a genome-wide microsatellite analysis. Subsequent work applying a locus counting approach has however suggested that in most cases LOD scores of greater than about 1.5 will arise only once by chance in a ‘real world’ 10 cM-density genome scan with some unavailability of parental genotyping and a missing data rate of ≈15%. In such a scenario, LOD scores of 2.80–2.88 (depending on the availability of parental data), rather than 3.63, equate to a genome-wide significance level of P = 0.05. We therefore report all loci giving LOD scores ≥ 1.5.
Among the 146 patients taking monotherapy for hypertension, 22% were treated with diuretics, 36% with beta-blockers, 32% with two drugs, and 5% were treated with three or more drugs. Among these, 826 individuals from 222 families had adequate quality echocardiographic data available. Of these, 826 individuals from 222 families had adequate quality echocardiographic data available.

There was no significant relationship between ECG or echo LVH and duration of hypertension in regression analyses, therefore no adjustment for this was made in the linkage analyses. Among the hypertensives with ECG and echo measurements, 25% were untreated, 45% were treated with one drug, 25% were treated with two drugs, and 5% were treated with three or more drugs. Among the 146 patients taking monotherapy for hypertension, 22% were treated with diuretics, 36% with beta-blockers, 32% with ACE-inhibitors (ACE-I), and 8% with calcium antagonists. Analysis of variance showed no significant heterogeneity in either echo or ECG LV mass between the drug classes in those patients on monotherapy, therefore no drug-specific adjustments were made in the linkage analyses.

A summary of LOD scores \( \geq 1.5 \) is presented in Table 2. There were no LOD scores attaining conventional genome-wide levels of significance on Lander-Kruglyak criteria (LOD \( \geq 3.63 \)).

There were three regions attaining or approaching suggestive evidence of linkage for particular measures of LVH (LOD \( \geq 2.20 \)). There was a region of suggestive linkage for ECG Sokolow-Lyon voltage on chromosome 10q23.1 (106.3 cM; LOD = 2.21, \( P = 0.0007 \)), for ECG Cornell voltage product on chromosome 17p13.3 (6 cM; LOD = 2.67, \( P = 0.0002 \)), and for ECG LV mass on chromosome 12q14.1 (78 cM; LOD = 2.19, \( P = 0.0007 \)). Of note, this last region (chromosome 12q14.1) also gave evidence of linkage to the ECG Cornell voltage product (LOD = 1.73, \( P = 0.002 \)). There were two further regions with weaker evidence of linkage for ECG Cornell voltage product on chromosomes 4 (211.9 cM; LOD 1.68, \( P = 0.003 \)) and 16 (7.6 cM; LOD = 1.85, \( P = 0.002 \)). ECG LV mass showed some evidence of linkage to chromosome 7 at 120 cM (LOD = 1.67, \( P = 0.003 \)). There was no significant linkage region for Cornell voltage, echocardiographic septal thickness in diastole or LV internal dimension in diastole.

### Table 1 Characteristics of the eligible study sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>362 (43.8)</td>
</tr>
<tr>
<td>Age in years, mean (± SD)</td>
<td>52.4 (13.5)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>349 (42.3)</td>
</tr>
<tr>
<td>Antihypertensive treatment, n (%)</td>
<td>330 (40.0)</td>
</tr>
<tr>
<td>Systolic BP, mean in mmHg (± SD)</td>
<td>137 (21)</td>
</tr>
<tr>
<td>Left ventricular hypertrophy, n (%)</td>
<td>86 (9.9) by ECG criteria</td>
</tr>
<tr>
<td>Weight, mean in kg (± SD)</td>
<td>76.8 (14.7)</td>
</tr>
<tr>
<td>Height, mean in m (± SD)</td>
<td>1.68 (0.1)</td>
</tr>
<tr>
<td>Body mass index, mean in kg/m² (± SD)</td>
<td>27.1 (4.8)</td>
</tr>
<tr>
<td>Waist-hip-ratio (± SD)</td>
<td>0.87 (0.1)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>23 (2.8)</td>
</tr>
</tbody>
</table>

Table shows data on 868 individuals from 224 families with ECG available. Of these, 826 individuals from 222 families had adequate quality echocardiographic data available.

### Discussion

We conducted a whole genome linkage scan for heritable electrocardiographic and echocardiographic measures of LVH, and found that the individual measures yielded distinct regions suggesting genetic linkage. We obtained four loci with LOD scores for Cornell voltage product above 1.5, while only one such LOD score might have been expected by chance alone. Thus, it is likely that some of the linked regions we describe harbour major loci influencing the Cornell voltage product. One of these four markers also gave evidence of linkage (LOD = 2.19) to the trait of ECG LV mass, which incorporates some overlapping primary ECG parameters (RaVL + SV₃), but adjusts these by body weight rather than QRS duration.

There are many ECG indices of LVH, and both the prevalence of LVH in the population and the relative risk of mortality in those classified as having LVH, varies depending on which index is adopted. Hsieh et al. showed that measures of LVH that incorporate criteria additional to QRS complex size (such as complex duration in the case of Cornell voltage product) are better predictors of cardiovascular mortality than criteria based on QRS voltage alone. The regions we have identified that are linked to Cornell voltage product may therefore be particularly interesting to examine for association with cardiovascular mortality in prospective studies. Sokolow-Lyon voltage appears to be influenced to a greater extent than Cornell voltage product by body habitus, with a tendency to underestimate LV mass in overweight or obese individuals. Our cohort was, as would be expected, given the selection criteria for hypertension, moderately overweight (mean BMI 27.1), and this may, in part, account for the lack of overlap between loci showing linkage to Cornell voltage product and Sokolow-Lyon voltage. However, the focus on chromosome 10 showing linkage to Sokolow-Lyon voltage may...
Few prior studies have evaluated linkage for cardiac hypertrophy in man.16 To the best of our knowledge, no linkage studies have been reported on electrocardiographic measures of cardiac hypertrophy. Prior analyses of echocardiographic measures of LVH in the Framingham Heart Study found a quantitative trait locus on chromosome 22 with a LOD score of 1.57 for echocardiographic LVH when compared with ECG, this initially seems paradoxical. However, an alternative explanation would be that electrical and anatomical measures of cardiac hypertrophy have separate genetic determinants, with different genetic architecture involving several major loci for electrical measures of hypertrophy, and no major loci for anatomical measures of hypertrophy. A different genetic basis would fit with the different underlying biology of these traits; ECG voltages reflect the cardiomyocyte compartment, whereas anatomical measures of LVH (such as echo LVH) encompass both cellular and interstitial components.

It remains to be established whether LVH measured by echocardiography LVH with LOD scores > 2.0 on chromosomes 10, 12, and 17 (Table 2). Positional candidate genes in these regions that are expressed in the heart include those encoding neuregulin-3 (NRG3) and growth hormone-inducible transmembrane protein (GHI1P) on chromosome 10, keratin hair basic 5 protein (KRTB5), heterochromatin protein 1 (HP1), integrin alpha-5 (ITGAS), low density lipoprotein-related protein 1 (LRP1) and ATP synthase beta polypeptide (ATP5B) on chromosome 12, and gem-associated protein 4 (GEMIN4), platelet-activating factor acetylhydrolase 1b alpha (PAFAH1B1), and replication protein A1 (RPA1) on chromosome 17.

Although only moderate LOD scores (1.6–2.67) were obtained in this study, there is precedent for the successful identification of susceptibility genes for complex diseases under linkage peaks of such magnitude. For example, ALOX5AP was successfully identified as a susceptibility gene for myocardial infarction (MI) and stroke in a linkage study conducted by Helgadottir et al. In that study of Icelandic families, moderate LOD scores (1.5–2.9) were obtained for a locus that was identified for MI and stroke when the data were divided on the basis of sex and age-of-onset. However, none of these statistics fulfilled the stringent criteria for genome-wide significance, which is not unusual for linkage studies of complex disease. In particular, it seems highly unlikely that the four loci with LOD scores > 1.5 that we observed for Cornell voltage product, including one locus with an LOD score of 2.67 and one that also showed linkage to ECG LV mass, all arose by chance.

This study has certain limitations. A substantial proportion of the hypertensive individuals in this study were taking antihypertensive treatment, and there was substantial heterogeneity present both with respect to agents and dosages. Attempting to adjust for each of the drug classes, combinations, and dosages would likely have been very imprecise. Accordingly, we adjusted our measurements of echo and ECG LVM for BP at the time of examination, as this to some extent took account both of the effect of hypertension on raising LV mass and the mitigating effect of concomitant treatment. However, it remains possible that the effect of treatment has tended to decrease the power of the study, and the

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Chromosomal location (cM)</th>
<th>Flanking microsatellite marker</th>
<th>LOD score</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECG Sokolow-Lyon voltage</td>
<td>Chromosome 10 10p23.1 (106.3)</td>
<td>D10S1686</td>
<td>2.21</td>
<td>0.0007</td>
</tr>
<tr>
<td>ECG Cornell voltage product</td>
<td>Chromosome 4 4q35.2 (211.9)</td>
<td>D45426</td>
<td>1.68</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Chromosome 12 12q14.1 (78)</td>
<td>D12S83</td>
<td>1.73</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Chromosome 16 16p13.2 (7.6)</td>
<td>D16S404</td>
<td>1.85</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Chromosome 17 17p13.3 (6)</td>
<td>D17S831</td>
<td>2.67</td>
<td>0.0002</td>
</tr>
<tr>
<td>ECG LV mass</td>
<td>Chromosome 7 7q22.1 (120)</td>
<td>D7S515</td>
<td>1.67</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Chromosome 12 12q14.1 (78)</td>
<td>D12S83</td>
<td>2.19</td>
<td>0.0007</td>
</tr>
<tr>
<td>Echo left ventricular mass</td>
<td>Chromosome 5 5p14.1 (40.3)</td>
<td>D5S419</td>
<td>1.6</td>
<td>0.003</td>
</tr>
</tbody>
</table>
LOD scores we present may therefore be somewhat underestimated. Our method should not, however, have biased our results towards false-positive findings.

To the best of our knowledge, we report the first whole genome analysis of electrocardiographic measures of LVH and we provide suggestive evidence of linkage to several quantitative trait loci. The electrocardiographic measures showed greater evidence of linkage than measures for echocardiographic cardiac hypertrophy. This study lays the basis for future association studies and positional cloning of genes that influence electrocardiographic LVH, a relatively unexplored trait despite its independent prognostic significance.

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Conflict of interest

None declared.

References


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