Major Quantitative Trait Locus for Resting Heart Rate Maps to a Region on Chromosome 4

Lisa J. Martin, Anthony G. Comuzzie, Gabriele E. Sonnenberg, Joel Myklebust, Roland James, Jacqueline Marks, John Blangero, Ahmed H. Kissebah

Abstract—Multiple studies have identified resting heart rate as a risk factor for cardiovascular disease independent of other cardiovascular disease risk factors (such as dyslipidemia and hypertension). Previous studies have examined heart rate in hypertensive individuals, but little is known about the genetic determination of resting heart rate in a normal population. Therefore, our objective was to perform a genome screen on a population containing normotensive and hypertensive individuals. We performed variance decomposition linkage analysis using maximum likelihood methods at ≈10 cM intervals in 2209 individuals of predominantly North European ancestry. We estimated the heritability of resting heart rate to be 26% and obtained significant evidence of linkage (logarithm of the odds [LOD]=3.9) for resting heart rate on chromosome 4q. This signal is in the same region as a quantitative trait locus (QTL) for long QT syndrome and a QTL for heart rate in rats. Within the 1-LOD unit support interval, there are 2 strong candidates: ankyrin-B and myozenin 2. (Hypertension. 2004;43:1146-1151.)

Key Words: genetics ■ pulse ■ cardiovascular diseases ■ human

Cardiovascular disease (CVD) has reached epidemic proportions. In 2000, CVD was the leading cause of death in the United States, accounting for 36.4% of deaths.1 Risk factors associated with CVD include obesity, dyslipidemia, diabetes, hypertension, smoking, and a sedentary lifestyle.2 However, resting heart rate is often overlooked as a risk factor, even though multiple studies have identified resting heart rate as a CVD risk factor.3–11 Moreover, although associated with other CVD risk factors, resting heart rate is an independent predictor of CVD.3,6,12

Given the potential clinical significance of resting heart rate, mechanisms of heart rate control are of great biomedical interest. In brief, a heartbeat begins with a small group of specialized muscle cells in the sinoatrial node. These cells generate electrical signals that spread throughout the heart and cause it to contract with a regular, steady beat. Although the sinoatrial node generates the heartbeat, heart rate is under tight neurohumoral control.

Interestingly, genetic factors also influence heart rate. Heart rate has been demonstrated to have a significant genetic component of variation.13 Additionally, quantitative trait locus or loci (QTL) for heart rate have been identified in drosophila,14 mice,15 rats,16–18 and humans.19 However, most genetic studies have used hypertensive subjects. Given that heart rate is associated with blood pressure,3,8,10 it is unclear whether the QTL are applicable to the normal variation in heart rate or are specific to elevated blood pressure. Therefore, our objective was to perform a genome screen on a population not ascertained on hypertensive status. Furthermore, because heart rate has been associated with CVD risk factors, we will examine the relationship between heart rate and CVD risk factors in a subset of unrelated individuals.

Methods

Subjects

Subjects were participants of the Metabolic Risk Complications of Obesity Genes project, which recruited participants and their families from Take Off Pounds Sensibly, Inc (TOPS) membership in the midwestern United States.20 Research protocols were approved by the Institutional Review Board of the Medical College of Wisconsin.

In this study, 2209 individuals distributed across 411 white families of predominantly northern European ancestry participated. Exclusion criteria for these analyses included: pregnancy; use of β-blockers; type 1 diabetes mellitus; history of cancer, renal or hepatic disease; and heart rate observations >4 SD from the mean.

Heart Rate

Heart rate was measured in triplicate after participants had rested for 15 minutes. Heart rate was measured by palpating the radial pulse by an experienced practitioner for 60 seconds. Repeatability of heart rate measurement varied by <5%. The mean of the second and third measurements was used for analysis.

Genotyping

DNA was extracted from whole blood using Puregene kits (Gentra Systems, Minneapolis, Minn); 366 autosomal markers were geno-
typed at Marshfield Medical Research Foundation using Weber screening set 9 (Research Genetics, Huntsville, Ala.). Samples were analyzed by automated high-throughput scanning fluorescence detectors.20 Genotypes inconsistent with Mendelian inheritance were removed. The average (±SD) heterozygosity of these markers was 0.79±0.06, and the sex-averaged genetic spacing was 9.1±3.8 cM.

Statistical Methods

Linkage

Variance components analysis was used to test for linkage using SOLAR.21–23 Briefly, an extension of the strategy developed by Amos21 was used to estimate the genetic variance attributable to a specific chromosomal location by specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent (IBD) relationships at a given marker locus.22 We tested the null hypothesis that the additive genetic variance caused by a QTL ($\sigma_q^2$) equals zero (no linkage) by comparing the likelihood of the restricted model with that of a model in which $\sigma_q^2$ is estimated.24 The difference between the 2 log 10 likelihoods produces a logarithm of odds (LOD) score equivalent to the classical LOD score.25,26 An evaluation of LOD score was performed every centiMorgan along the autosomal chromosomes, marker distances determined using CRI-MAP.27 Genome-wide significance was determined using Feinberg functions for decay in correlation across the genome based on LOD score.25,26

Relationship Between Heart Rate and CVD Risk Factors

To determine whether elevated heart rate is associated with CVD risk factors, we classified individuals as having normal (measures within 0.5 SDs from the mean) or elevated heart rate (measures >2 SDs from the mean). To obtain an unrelated sample, 1 family member was chosen at random. One-way $t$ tests were used to determine if the elevated group displayed a more negative risk profile.

Results

After cleaning the data, 2031 individuals from 409 families were available for analysis. Heart rate was normally distributed with a kurtosis of 0.61, which is within the acceptable range for the analytical methods used.26 Table 1 shows the pairwise relationships represented by these data. Table 2 reports the mean levels for CVD risk factors in this population.

To account for environmental variation, we included age, sex, smoking, menstrual status, intake of estrogen or birth control steroids, history of asthma, history of diabetes, and diabetes, hypertensive, and cholesterol medication usage as covariates. We included body mass index (BMI) and hypertensive status (systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or using high blood pressure medication) as covariates but they did not substantially influence the results; therefore, analyses with BMI and hypertensive status are not reported.

Linkage

In the additive genetic model (no linkage), covariates explained 5.6% of the phenotypic variability in heart rate. Significant covariates were sex, asthma, smoking status, diabetes medications, and antihypertensive medications. Of these covariates, only antihypertensive medication usage was associated with reduced heart rate (Table 3). The heritability of heart rate was estimated at 0.26±0.05 ($P=0.0000001$), demonstrating a significant genetic component of variation.

Figure 1 displays the results by chromosome from the linkage analysis. Between markers D4S2623 and D4S1644, a LOD unit support interval spans from 121 to 135 cM from pter (Figure 2). The flanking markers have been physically mapped to 111.2 Mb (D4S2623) and 142.1 Mb (D4S1644) on chromosome 4.29

| TABLE 1. Distribution of Relative Pairs Used in the Multipoint Linkage Analysis |
|-----------------------------|-----------------------------|
| Relationship                | Individuals (n)             |
| Parent–offspring            | 1780                        |
| Siblings                    | 1953                        |
| Grandparent–grandchild      | 70                          |
| Avuncular                   | 373                         |
| Half siblings               | 55                          |
| Half avuncular              | 6                           |
| First cousins               | 181                         |
| First cousins, once removed | 2                           |
| Identical sib pair          | 8                           |

| TABLE 2. Cardiovascular Disease Risk Factors in Males and Females in the Full Sample |
|-----------------------------------|--------|--------|
| n                                 | Males  | Females|
| Age (y)                           | 52.02±0.77 | 46.93±0.36 |
| Pulse (beats/min)                 | 70.33±0.46 | 73.23±0.24 |
| HDL (mg/dL)                       | 34.11±0.46 | 40.13±0.29 |
| LDL (mg/dL)                       | 111.19±1.58 | 123.47±1.03 |
| Cholesterol (mg/dL)               | 197.27±1.93 | 198.52±1.14 |
| Triglycerides (mg/dL)             | 136.90±4.38 | 128.05±2.33 |
| Insulin (pmol/L)                  | 94.05±3.35 | 102.94±2.19 |
| Glucose (mg/dL)                   | 92.42±1.43 | 91.53±0.82 |
| Waist (cm)                        | 103.06±0.71 | 103.18±0.52 |
| Systolic BP (mm)                  | 131.63±0.75 | 128.95±0.47 |
| Diastolic BP (mm)                 | 81.83±0.44  | 80.60±0.27  |
| BMI (kg/m²)                       | 28.74±0.25  | 33.33±0.21  |

BP indicates blood pressure; BMI, body mass index.
Relationship Between Heart Rate and CVD Risk Factors

To determine whether elevated heart rate was associated with CVD risk factors, we identified individuals with normal and elevated heart rate. Because some CVD risk factors differ by sex, we analyzed the sexes separately, resulting in 206 and 111 females and males with normal heart rate and 32 and 11 females and males with elevated heart rate, respectively. The male group had substantially lower numbers, which can be largely attributed to the fewer number of males, because females outnumbered the males 3 to 1 in the larger sample. The male group was underpowered to detect phenotypic differences; therefore, only female results are reported (Table 4). To account for multiple comparisons, we used Bonferroni correction (0.05/number of tests\[10\]=0.005) to determine statistical significance.

The ranges of normal and elevated heart rates were 71 to 75 and 92 to 112 bpm, respectively. Individuals with elevated heart rate exhibited significantly elevated insulin and glucose levels, waist circumference, BMI, and diastolic blood pressure (P<0.005) and suggestively elevated triglyceride levels and systolic blood pressure (P<0.01). Males exhibited similar trends except for BMI and glucose.

Discussion

Resting heart rate is a clinically important variable that is associated with CVD, atherosclerosis, and outcome after myocardial infarction. Moreover, individuals with tachycardia have elevated cholesterol, triglycerides, insulin, glucose levels, BMI, and systolic and diastolic blood pressure. However, it is unclear whether this relationship persists in normal populations. Therefore, we were interested whether elevated heart rate was associated with CVD risk factors. Our analyses demonstrated that females with elevated heart rate exhibited significantly elevated insulin and glucose levels, waist circumference, BMI, and diastolic blood pressure (P<0.005) and suggestively elevated triglyceride levels and systolic blood pressure (P<0.01), thereby supporting previous research and suggesting clinical importance.

Given the clinical importance of resting heart rate, it is important to identify determinants of resting heart rate.

Table 4. Comparison of Cardiovascular Disease Risk Factors in Females With Elevated Heart Rate (92 to 112 beats/min) and Females With Normal Heart Rate (71 to 75 beats/min)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Elevated Heart Rate</th>
<th>Normal Heart Rate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>32</td>
<td>206</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>48.0±2.8</td>
<td>49.1±1.0</td>
<td>0.3467</td>
</tr>
<tr>
<td>Pulse (beats/min)</td>
<td>100.0±0.8</td>
<td>72.3±0.1</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>37.9±1.8</td>
<td>40.7±0.8</td>
<td>0.0886</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>140.0±9.0</td>
<td>123.3±2.7</td>
<td>0.0143</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>211.5±8.9</td>
<td>198.5±3.0</td>
<td>0.0609</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>147.0±12.2</td>
<td>119.4±4.2</td>
<td>0.0092</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>166.2±23.2</td>
<td>102.2±5.8</td>
<td>0.00015</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>109.1±9.4</td>
<td>91.6±2.0</td>
<td>0.0030</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>113.8±4.9</td>
<td>103.2±1.3</td>
<td>0.0026</td>
</tr>
<tr>
<td>Systolic BP (mm)</td>
<td>139.1±4.2</td>
<td>129.4±1.4</td>
<td>0.0056</td>
</tr>
<tr>
<td>Diastolic BP (mm)</td>
<td>90.5±2.1</td>
<td>80.0±0.7</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>37.8±2.3</td>
<td>33.0±0.5</td>
<td>0.0010</td>
</tr>
</tbody>
</table>

P value of 0.005 is considered significant.
BP indicates blood pressure; BMI, body mass index.
Previous studies have demonstrated that resting heart rate in hypertensive individuals is under genetic control. However, our goal was to identify the genetic determinants of resting heart rate in a population not ascertained on hypertensive status. In this study, 44.4% of the individuals were classified as hypertensive. This prevalence is slightly higher than the United States average, 32.2%; however, given that our sample was ascertained for obese individuals, the elevated BMI (mean=32.01) may explain the increased prevalence. Nonetheless, individuals using β-blocker medications (n=4) were excluded.

We estimated the heritability of resting heart rate to be 26%, which is similar to Singh et al’s estimate of 21% but is lower than Jedrusik et al’s estimate of 59% in twins. However, twin studies have often been recognized as producing inflated heritability estimates. Interestingly, other heart rate phenotypes have also been demonstrated to have significant genetic components of variation, including response to training and heart rate variability. All of these data support a moderate genetic contribution to heart rate.

Our linkage results suggest that a gene located near 4q25-q27 is responsible for the variability in resting heart rate. This marker has been physically mapped to 130.6 Mb, cytogenetic location 4q28.2. A previous genome screen of resting heart rate also identified a QTL on chromosome 4, but our signal maps 55.2 Mb from these markers, suggesting the signals may be caused by different genes. However, errors in mapping or low information content may cause poor localization of either or both signals; thus, these could be reflecting the same underlying genetic effect. Our signal does localize to the same region as the QTL for type 4 long QT syndrome, a syndrome associated with bradycardia (slow heart rate). Additionally, our signal localizes proximal to a homologous region identified in the rat, which contains a putative heart rate gene (Figure 3). Using Ensembl homology maps, the flanking markers from our chromosome 4 QTL overlap with the markers for the rat chromosome 2 QTL. Because heart rate is correlated with measures of adiposity, insulin sensitivity, and blood pressure, we also were interested to determine whether our signal localized to regions with QTL for these phenotypes. In our population, 2 phenotypes yield nominal linkage in this region: systolic blood pressure (LOD=1.8) and leptin (LOD=1.0). Moreover, suggestive linkages have been reported in the 4q21-4q32 region for abdominal subcutaneous fat, insulin, and blood pressure. The consistency across populations and related phenotypes suggest that this may be an important locus.

There are 2 interesting candidate genes within our support interval (Figure 4), ankyrin-B (ANKB) and myozenin 2. By fluorescence in situ hybridization, ANKB has been assigned to 4q25-q27. Ankyrin promotes targeting of ion channels to the proper membranes in cells. Interestingly, type 4 long QT syndrome has been attributed to an A to G mutation at position 4274 (E1425G) in ANKB. Mice heterozygous for this mutation exhibited abnormalities in calcium homeostasis in cardiac muscle cells, bradycardia, and increased sudden cardiac death associated with physical exertion or stress.

Given the importance of ankyrin-B in cardiac signaling, it is possible that mutations in ANKB could influence heart rate.

MYOZ2 was localized to chromosome 4q26-q27 using fluorescence in situ hybridization. MYOZ2 belongs to a gene family of calsarcins and is expressed in adult cardiac tissue. Calsarcins couple muscle activity to calcineurin (a calcium/calmodulin-dependent serine-threonine phosphatase in cardiac tissue) activation. Calcium signaling is a critical component of the excitation-contraction coupling necessary for cardiac activity. Moreover, alterations in intracellular calcium may influence sinoatrial pacemaking through hyperpolarization-activated current, and calcineurin contributes to calcium homeostasis by modulating the ATPase activity of Ca²⁺ pumps. Given the role of MYOZ2 in the regulation of calcineurin activation, it may indirectly influence calcium signaling and pacemaker function.

Although we have identified a QTL for resting heart, the effects of excess adiposity, hypertension, and hypertension medication usage may impact our ability to detect genes. However, the signal on chromosome 4 persisted with the inclusion of hypertensive status and BMI as covariates, albeit the signal was reduced. Additionally, because hypertensive medications may influence resting heart rate, individuals using these medications were excluded and our signal was only slightly attenuated. These results demonstrate the robust nature of our signal and suggest that these findings are generally applicable.
Genotyping was funded by the Mammalian Genotyping Service at the Marshfield Medical Research Foundation. TOPS, Inc provided funds for establishment of the family database, phenotyping, and linkage analysis. Phenotyping costs were provided in part through a collaborative research agreement with Millennium Pharmaceuticals, Inc. We thank Dr D. Woodrow Benson and Dr Ulrich Broeckel for their helpful comments.

References

Perspectives
Given the clinical importance of resting heart rate, it is important to identify determinants of resting heart rate. In a population predominantly of Northern European ancestry recruited via an obese proband, we estimated the heritability of resting heart rate to be 26%. Moreover, we obtained significant evidence of linkage (LOD = 3.9) for resting heart rate on chromosome 4q. This signal is in the same region as a QTL for long QT syndrome 435 and a QTL for heart rate in rats.16 Within the 1 LOD unit support interval, there are 2 strong candidates: ANKB and MYOZ2. Given the biological support for our 2 positional candidate genes and the strength of our LOD score (LOD = 3.9), our goal is to further explore this linkage signal by typing single nucleotide polymorphisms in both candidate genes. By typing single nucleotide polymorphisms in these candidate genes, we hope to identify functional polymorphisms accounting for heart rate variability. Because obese individuals usually exhibit autonomic dysfunction, such as heart rate regulation abnormalities, and because this dysfunction is not expressed in all obese individuals, identification of functional polymorphisms in heart rate candidate genes should help in identifying those individuals with this disorder with increased CVD risk. Efforts can therefore be directed to prevent this serious health complication.

Acknowledgments
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Figure 4. Localization of the positional candidate genes in reference to the markers flanking the maximum LOD score.
29. Ensembl Genome Data Resources. Available at: http://www.sanger.ac.uk.
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