Polymorphisms in multiple genes are associated with resting heart rate in a stepwise allele-dependent manner

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OBJECTIVE The purpose of this study was to use a candidate gene approach to identify common polymorphisms that are associated with resting sinus heart rate in a population without overt cardiovascular disease.

BACKGROUND Increased resting heart rate is significantly associated with susceptibility to development of myocardial infarction, sudden cardiac death, and overall cardiac mortality.

METHODS A longitudinal cohort of 1468 individuals (active and retired middle-aged Canadian firefighters) who were enrolled in the Firefighters and Their Endothelium (FATE) study was evaluated. Resting heart rate was recorded from the electrocardiogram (ECG) obtained at enrollment. Candidate genes were selected for their known roles in sinus node automaticity and/or its regulation, and single nucleotide polymorphisms (SNPs) with a minor allele frequency of $\geq$0.20 were targeted. A total of 53 SNPs in 46 genes were selected and analyzed in a screening sample, and 33 SNPs in 29 genes were evaluated in the full population.

RESULTS Univariate analysis detected five putative associations between HR and SNPs. As expected, environmental covariates were identified. Three polymorphisms, ADRB1 G389R, SCN5a H558R, and CASQ1 intron 2, remained statistically significant and independent of covariates. Some alleles were associated with higher and some with lower heart rates. A stepwise increase in heart rate was observed that was dependent on the number of tachycardia-associated alleles with progressive increases in mean heart rate from 51 to 66 bpm.

CONCLUSIONS Common polymorphisms are associated with heart rate in a stepwise allele-dependent manner.

KEYWORDS Heart rate; Single nucleotide polymorphism; Cardiac sodium channel; Beta-1 adrenergic receptor; Calsequestrin; Cohort study

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Introduction

Increased resting heart rate is associated with the development of important cardiovascular outcomes including myocardial infarction, sudden cardiac death, and cardiovascular mortality as well as overall mortality in the general population.[1–8] This relationship is independent of other traditional risk factors for cardiovascular disease and physical fitness.[9,10]

Control of resting heart rate (HR) is complex. Sinoatrial HR is regulated by the interaction of genetic, environmental, autonomic, and pathophysiologic factors.[11] Investigators from the Framingham study and others have previously demonstrated that the genetic contribution to variance in HR is between 21% and 26%.[12–14] The advent of genomic science has led to the use of single nucleotide polymorphism (SNP) analysis to identify potential genetic contributors to resting sinus HR. Many have focused on the beta-adrenergic receptor, types 1 and 2, given its central role in autonomic regulation of HR. Results have been mixed—some investigators have found an association between SNPs in these genes,[15–17] which have not always been replicated in other populations and conditions.[18–20] Most of these studies have genotyped SNPs in one or two genes, and none has attempted a more detailed assessment of associations between polymorphisms in nonadrenergic genes and resting HR. We also reasoned that a relatively large sample of subjects would be required to address the issue of the physiologic effects associated with the simultaneous presence of multiple independently segregated alleles, each contributing to the physiologic outcome. Accordingly, our first objective was to identify common SNPs in candidate genes associated with variation in resting HR in a relatively large population without overt cardiovascular disease. We then assessed the physiologic outcomes of the simultaneous presence of independent tachycardia-associated alleles.

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Methods

Study population

The study population consisted of participants in the Firefighters and Their Endothelium (FATE) study, a longitudi-

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nal cohort trial designed to assess the predictive value of brachial artery flow-mediated vasodilatation in relation to cardiovascular endpoints. The cohort included active and retired middle-aged Canadian firefighters. The details of its design have been published.[21] Exclusion criteria included a documented history of coronary artery disease, peripheral vascular disease, or cerebrovascular disease. Recruiting sites included Calgary and Red Deer, Alberta; Hamilton, Ontario; and Montreal, Quebec, all in Canada. Informed consent was obtained from all participants. The study was approved by the local ethics committee at each site.

Resting HR was measured from a 12-lead electrocardiogram (ECG) obtained at study enrollment. The ECG was recorded at rest during the initial visit. Two blinded investigators performed manual ECG analysis, and interobserver agreement on measurements was assured after overreading on a randomly selected sample of ECGs. Before the visit, participants were instructed not to ingest cardiac medications for a period of at least 5 half-lives and to abstain from alcohol, caffeine, and nicotine for at least 24 hours. A detailed medical history and limited physical examination were performed to document the presence of cardiovascular risk factors. Blood work was drawn for lipid levels and genetic testing.

Selection of polymorphisms

A candidate gene approach was used herein. Genes were selected for their known roles in (1) regulation of sinus node automaticity or conduction (cardiac ion channels), (2) Ca^{2+} homeostasis, (3) cardiac signal transduction or excitation-contraction coupling, (4) autonomic nervous system, (5) blood pressure regulation, (6) cardiac inflammation or thrombosis, (7) endothelial function, or (8) cardiac hypertrophy. After a literature review, and with the use of public databases, including PubMed and Online Mendelian Inheritance in Man, we selected SNPs within these genes. Criteria for selection were that the SNP lay in the coding (exon) or promoter regions of the gene and had a minor allele frequency of $\geq$0.20 or had been previously associated with an outcome of interest.

Genotyping of polymorphisms

All analysis occurred at the Molecular Diagnostics Laboratory at the Alberta Children's Hospital in Calgary. Genomic DNA was extracted from peripheral blood using a phenol chloroform extraction. At the outset of the study, participants were genotyped for 10 SNPs using the Applied Biosystems SNaPShot Multiplex System (Applied Biosystems, Foster City CA). Template DNA was prepared by three multiplex polymerase chain reaction (PCR) amplifications. The PCR products were pooled, and all 10 variant sites genotyped within one single nucleotide primer extension method (SNaPshot reaction). Denatured SNaPshot reactions were run on an ABI 377 8% polyacrylamide slab gel (Applied Biosystems), and the resulting genotypes were analyzed based on color and peak location.

The other polymorphisms were genotyped through the TaqMan allelic discrimination methodology (Applied Biosystems). The primer-probe sets were designed on Primer Express 2.0 according to the manufacturer's guidelines or ordered through Applied Biosystems' Assays-By Design service. Samples were amplified under the standard TaqMan reaction conditions recommended by the manufacturer. After amplification, automated genotype calling was carried out on the ABI PRISM 7900 (Applied Biosystems) Sequence Detection System. Controls for each population were verified through sequencing with Big Dye v3.1 (Applied Biosystems) on an ABI 377 8% polyacrylamide slab gel (Applied Biosystems).

Statistical analysis

Data are described as mean $\pm$ standard deviation (SD). Pearson correlations were performed to determine interobserver agreement for HR measurement and to compare HR by ECG versus pulse rate by physical exam. SNPs were tested for association by linear regression analysis using HR as the dependent variable. Significance levels were determined using both 1 and 2 degree of freedom genetic models. In the 1 degree of freedom model, the SNP's genotype was coded 0, 1, or 2 by counting the number of minor alleles, assuming codominance, with identical trait increases between genotypes. This test was the primary test used and has a relatively higher power to detect weak effects. In the other model, an SNP was transformed into two dummy variables representing the two possible genotypic changes. The first dummy variable was coded 1 for heterozygotes (Aa) and 0 otherwise. The second dummy variable was coded 1 only for individuals homozygous for the minor allele (aa). Both variables were included into a bivariable regression to quantify each genotype's effect, and a Wald procedure was performed to test the difference in effect between alleles. The average trait change per allele was calculated as the slope coefficient from the 1 degree of freedom model, and the variance attributable to an SNP was calculated as the adjusted R^2 value from the bivariable regression analysis.

For SNPs associated with HR at a two-sided significance level of $P < .05$ in the codominant model, we performed multivariable linear regression analysis to adjust for potential environmental confounders. Variables included in the final models were age, smoking status, hypertension, body mass index (BMI), diabetes, family history of vascular disease, and cardiovascular medications [aspirin, angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, beta-blockers, and calcium channel blockers].

To determine the significance of each SNP when controlled for the others, we generated a multivariable linear model incorporating the genotypic changes of all SNPs significant in the univariable analysis. To determine combined effects, we counted the number of alleles significantly associated with faster HR in each person to give an HR score and performed nonparametric trend analysis using the method of Cuzick,[22] with the score as the independent variable. Multiplicative terms for each two-SNP combina-