

Abstract

Cardiovascular disease (CVD) contributes to morbidity and mortality in type 2 diabetes (T2DM), but the relationship between T2DM and CVD is poorly understood. Electrocardiograph (ECG) intervals are predictors of CVD risk, and ECG interval abnormalities are often present at T2DM diagnosis. The Diabetes Heart Study has recruited families enriched for T2DM and phenotyped participants for subclinical measures of atherosclerosis and CVD risk factors. DNA from 1059 subjects (886 European American: EA, 173 African American: AA) were included in a genome scan for ECG intervals using microsatellite markers at an average spacing of 6.7cM. Variance component quantitative trait linkage analysis was performed adjusting for age, gender, ethnicity, BMI, T2DM status, smoking, and use of lipid-lowering medications. The strongest evidence for linkage was observed with QT interval (QTi) and markers on chromosome 4p15.31-p15.33 (LOD=2.45, LOD-1 interval 22cM-39cM). This evidence decreased to LOD= 1.51 when limited to EA subjects. While none of the Long QT Syndrome genes are located within the linkage support interval, the potassium channel interacting protein 4 (*KCNIP4*) gene on 4p15.3 is a strong positional candidate. *KCNIP4* modulates the activity of potassium channels involved in shaping the action potential of the heart. In EA families, restricting the analysis to T2DM subjects, linkage to QTi was observed on chromosome 2p13.2-p14 (LOD=1.62, LOD-1 interval 64cM-115cM). Candidate genes within this linked region include calcineurin B type 1 (*CNB1*), involved in heart valve morphogenesis and vessel assembly, and bone morphogenetic protein 10 (*BMP10*), involved in trabeculation of the embryonic heart. Evidence of linkage was also observed on chromosome 11p11.2-p13 with QRS interval in EA families (LOD=2.51, LOD-1 interval 32cM-58cM) and on chromosome 19q13.11-q13.43 in AA families (LOD=2.00, LOD-1 interval 64cM-93cM). The identification of genes contributing to variation in ECG intervals will help elucidate the role of these intervals in predicting CVD risk in persons with T2DM.

Background

An electrocardiogram (ECG) records the timing and pattern of intracardiac conduction and repolarization. The duration of, and interval between, the major elements of the ECG are influenced by physiologic and pharmacologic conditions, various myocardial diseases, and body habitus. Genetic factors also appear to influence cardiac conduction. For example, there are several rare monogenic disorders that lead to alterations in the QT interval.

Compared to rare monogenic disorders, there is less information about the extent to which common genetic variants contribute to population-wide variation in ECG phenotypes. Twin studies have inconsistently provided evidence of the heritability of heart rate and various ECG intervals. Unfortunately, most of these studies have been limited by modest sample sizes as well as the strengths and weaknesses of a twin study design. Thus, it is prudent that the genetic architecture of ECG intervals and heart rate be explored under alternative designs and in a large sample.

Clarifying the extent to which ECG phenotypes are influenced by genetic factors and identifying specific genetic variants involved could enhance our understanding of myocardial conduction and facilitate the development of new clinical modalities targeting these genes, or their products, to achieve beneficial clinical effects. In addition, knowing that a subject has a genetic predisposition to faster or slower conduction in various parts of the cardiac conduction system or myocardium could be useful when contemplating pharmacologic interventions with known effects on these same properties.

Methods

Study Design

The Diabetes Heart Study is being conducted in Forsyth County, North Carolina to study the genetic and epidemiological origins of cardiovascular disease in families affected with T2DM. Siblings concordant for diabetes were recruited from internal medicine clinics, endocrinology clinics, and community advertising. T2DM was defined as a clinical diagnosis of diabetes after the age of 34 years, in the absence of historical evidence of diabetic ketoacidosis. Unaffected siblings, similar in age to the siblings with T2DM, were also invited to participate, as were any additional diabetes-affected siblings. The sample includes European American and African American (approximately 18% of the total) participants. Recruitment was based upon family structure, and there were no inclusions/exclusions based on prior or current evidence of prevalent CVD at the time of recruitment.

The participant examinations were conducted in the General Clinical Research Center of the Wake Forest University Baptist Medical Center and included interviews for medical history and health behaviors, anthropometric measures, resting blood pressure, a 12-lead electrocardiogram, a fasting blood draw and a spot urine collection. Laboratory assays included urine albumin and creatinine, lipids, glycated hemoglobin, fasting glucose and blood chemistries. A detailed medical history was collected with emphasis on CVD history, procedures, etc.

Electrocardiogram

A resting 12-lead electrocardiogram (ECG) was performed to assess history of clinically significant (past or present) cardiovascular disease. All ECGs were performed in the General Clinical Research Center at Wake Forest University Baptist Medical Center and were read to detect clinical or subclinical evidence of coronary heart disease. ECGs were recorded on a Marquette ECG machine and transmitted to the ECG processing stations of the EPICARE center at Wake Forest University for coding, following standardized, extensively tested procedures. Prevalent ECG abnormalities are classified according to the Minnesota Code. Excluded from these analyses are individuals who self-reported myocardial infarction or who were identified by Minnesota Code as having had a myocardial infarction.

Genotyping

A genome-wide scan was conducted by the Mammalian Genotyping Service (MGS, Marshfield, WI) using Marshfield Microsatellite Set 13 at a mean inter-marker distance of 9.3 cM. The results reported here are from 886 European Americans (378 families) and 173 African Americans (75 families).

Linkage Analysis

LOKI was used to perform identity-by-descent (IBD) estimates based on ethnic-specific allele frequencies. Multipoint nonparametric QTL linkage analysis, as implemented in SOLAR, was performed on heart rate, PR interval, QRS interval, and QT interval. Covariates used in the linkage analysis included age, gender, ethnicity, body mass index (BMI), T2DM affection status, smoking status, and use of lipid-lowering medications. To better approximate the distributional assumptions of conditional normality and homogeneity of variance, PR interval, QRS interval, and QT interval were natural log transformed. Heart rate was square root transformed.

Results

Table 1. Demographics and ECG interval characteristics of the DHS sample.

Trait	African Americans		European Americans	
	Diabetic (n=156)	Non-Diabetic (n=17)	Diabetic (n=733)	Non-Diabetic (n=153)
Age	58.6±8.8 (58.2)	55.4±9.7 (56.4)	61.8±9.2 (61.6)	59.4±10.4 (59.4)
Female (%)	69.7	76.4	54.6	65.4
Years since diagnosis	10.7±8.0 (8.0)	-	10.4±7.3 (8.0)	-
HbA1c	8.9±2.74 (8.1)	6.71±0.61 (6.6)	7.71±1.83 (7.4)	6.57±0.63 (6.50)
BMI	34.0±7.3 (33.2)	32.2±6.6 (31.9)	32.6±7.0 (31.4)	28.8±5.2 (28.0)
ECG Trait				
Heart rate (bpm)	72.3±11.9 (72)	63.5±11.0 (59)	71.1±11.9 (70)	65.8±11.1 (65)
PR (msec)	164.0±23.9 (160)	171.3±29.3 (168)	165.1±24.1 (164)	160.4±22.6 (158)
QRS (msec)	89.4±12.0 (87)	88.0±10.7 (86)	91.0±10.9 (92)	89.5±10.8 (88)
QT (msec)	392.9±33.2 (390)	404.9±40.2 (408)	392.2±32.7 (390)	400.0±31.2 (398)

Figure 2. Genes located within the chromosome 4 LOD-1 support interval for QT interval.



Figure 1. QTL linkage analysis of QT interval. Marker locations are shown across the top and map position in cM is shown along the bottom.

QTL Linkage Analysis of QT Interval Chromosome 4

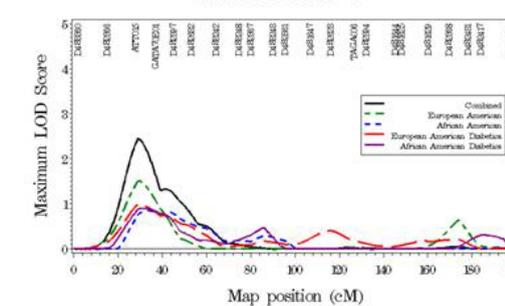


Table 2. Priority linkage peaks (LOD≥2.00) for heart rate and ECG intervals in the DHS sample.

Chromosome	Position	LOD	LOD-1 Interval	Flanking Markers	Trait	Sample
11	44 cM	2.51	32 cM-58 cM	D11S1392/D11S1993	QRS Interval	EA
4	29 cM	2.45	22 cM-39 cM	AT1015/GAT470E01	QT Interval	Combined
19	82 cM	2.00	64 cM-93 cM	D19S245/D19S254	QRS Interval	AA

Conclusions

In a comprehensively phenotyped collection of families enriched for T2DM, we have observed evidence for linkage of QT interval to loci on chromosome 4p15.31-p15.33, and QRS interval to loci on chromosome 11p11.2-p13 in EA families and chromosome 19q13.11-q13.43 in AA families. Determining genetic factors that influence ECG intervals in the general population has both clinical and research implications. For example, both heart rate and QT interval are known to be predictive of subsequent cardiovascular morbidity and mortality in healthy subjects independent of other known risk factors. Identifying variants within genes that contribute to population variations in these parameters may identify novel physiologic pathways that influence prognosis and provide additional screening tools for risk stratification. In a similar fashion, genetic variants that regulate myocardial hypertrophy and myocellular organization might contribute to population variations in the QRS interval. Knowing that certain subjects have a genetic predisposition for delayed conduction and repolarization might alter clinical decision-making concerning drugs that influence these parameters. The large number of well characterized families in the Diabetes Heart Study provides a unique resource for these types of genetic studies.