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Association of *NOS1AP* Genetic Variants With QT Interval Duration in Families From the Diabetes Heart Study

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Abstract

OBJECTIVES——Prolongation of the electrocardiographic QT interval is a risk factor for sudden cardiac death (SCD). Diabetic individuals are at increased risk for prolonged QT interval and SCD. We sought to replicate the finding that genetic variants in the nitric oxide synthase 1 adaptor protein (*NOS1AP*) gene are associated with QT interval duration in a type 2 diabetes–enriched sample of European ancestry.

RESEARCH DESIGN AND METHODS——Two single nucleotide polymorphisms (SNPs) in *NOS1AP* were genotyped in 624 European Americans and 127 African Americans from 400 pedigrees enriched for type 2 diabetes. An additive genetic model was tested for each SNP in ancestry-specific analyses in the total sample and the diabetic subset (European Americans, $n = 514$; African Americans, $n = 115$), excluding from the analyses individuals taking QT-altering medications.

RESULTS——In European Americans, rs10494366 minor homozygotes had a 9.3-ms-longer QT interval compared with major homozygotes ($P = 5.7 \times 10^{-5}$); rs10918594 minor homozygotes had a 12.5-ms-longer QT interval compared with major homozygotes ($P = 1.5 \times 10^{-6}$). Restricting analyses to the diabetic European Americans strengthened the effect despite the reduction in sample size (11.3-ms difference, $P = 5.1 \times 10^{-5}$; 13.9-ms difference, $P = 1.6 \times 10^{-6}$, respectively).

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No association between the *NOS1AP* SNPs and QT interval duration was observed in the limited number of African Americans.

CONCLUSIONS——Two *NOS1AP* SNPs are strongly associated with QT interval duration in a predominately diabetic European-American sample. Stronger effects of *NOS1AP* variants in diabetic individuals suggest that this patient subset may be particularly susceptible to genetic variants that influence myocardial depolarization and repolarization as manifest in the QT interval.

Sudden cardiac death (SCD) claims ~300,000 lives per year in the U.S. (1). Numerous community-based studies have established QT interval as an independent risk factor for SCD (2–5). Moreover, QT interval prolongation and resultant arrhythmia on exposure to cardiac and noncardiac medications is a major barrier to drug development (6). Genetic factors contribute significantly to both SCD risk and QT duration (7–10). However, with the exception of rare monogenic forms of congenital long or short QT syndromes, the precise genetic variants that account for the heritability of these traits in the general population have not been established.

Data from a recently staged genome-wide association study of 200 German women from the extremes of the QT interval distribution identified an association between common variants in the nitric oxide synthase 1 adaptor protein (*NOS1AP*) gene and adjusted QT interval duration (11). This association was replicated in two other population-based studies (11), an elderly population (12) and the genetically more homogeneous Old Order Amish population (13). However, the propensity for false-positive reports in genetic association studies warrants replication in additional cohorts: especially cohorts with high risk for prolonged QT interval and SCD due to competing factors, such as diabetes and coronary disease. In addition, because the common variants at the *NOS1AP* locus are highly differentiated across continental ancestral groups, the extension of findings into samples of non-European ancestry is important (14) (http://www.hapmap.org/cgi-perl/gbrowse/hapmap_B36/).

In the current study, we attempted to replicate the association of two previously implicated *NOS1AP* single nucleotide polymorphisms (SNPs), rs10494366 and rs10918594, with adjusted QT interval duration in the Diabetes Heart Study, a sample of European-American and African-American pedigrees enriched for type 2 diabetes. The extent to which QT interval is influenced by genetic variants will expand our understanding of myocardial electrical activity and could facilitate the development of new clinical therapies to reduce the risk of SCD.

RESEARCH DESIGN AND METHODS

The study sample consists of 624 European-American individuals (514 type 2 diabetes–affected individuals and 110 unaffected individuals from 331 families) and 127 African-American individuals (115 type 2 diabetes–affected individuals and 12 unaffected individuals from 69 families) from the Diabetes Heart Study. Ascertainment and recruitment have been described previously (15–17). Briefly, siblings concordant for type 2 diabetes and lacking advanced renal insufficiency were recruited. Type 2 diabetes was defined clinically as diabetes developing after the age of 35 years treated with insulin and/or oral agents, in the absence of historical evidence of ketoacidosis.

All protocols were approved by the institutional review board of Wake Forest University School of Medicine, and all participants gave informed consent. 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, fasting total cholesterol, electrocardiography, and spot urine collection. Not all measurements were available for all participants. Excluded from these analyses were 94 European-American individuals (9.5%) and 5 African-American individuals (2.8%) from the total Diabetes Heart Study population who were identified by the Minnesota Code (18) as having had a myocardial infarction. Individuals were scored as using QT-altering medications if they were on at least one of the following medications: *1*) a medication known to prolong QT interval as listed at http://qtdrugs.org (http://www.arizonacert.org/medical-pros/drug-lists/drug-lists.htm); *2*) digoxin (known to shorten QT interval duration); or *3*) hormone replacement therapy (personal communication, D.M.H., MD, MHS), resulting in the exclusion of 346 European-American individuals (34.7%) and 50 African-American individuals (28.1%) from analysis.

QT interval measurement.

A resting 12-lead electrocardiogram (ECG) was obtained for each study participant using a Marquette MAC 5000 ECG instrument (Marquette Electronics, Milwaukee, WI) using a standardized protocol with special attention to precise placement of the electrodes. All ECGs were performed in the General Clinical Research Center at Wake Forest University School of Medicine and were electronically transmitted to a central reading center (EPICARE). Each ECG was visually analyzed for recording errors and graded for quality. The ECGs were then analyzed using the Marquette 12-SL Program, 2001 version (GE Healthcare, Waukesha, WI) (19). QT interval duration was defined as the duration in milliseconds from the initial deflection of the QRS complex until the return of the T-wave to the electrical baseline. The QT duration was measured in each of the 12 ECG leads, and the longest QT duration was selected as the trait for analysis.

The heart rate–corrected QT interval (QTc) was calculated using Bazett's formula: $QTc = QT\sqrt{HR \neq 60}$ (20). The QTc is commonly interpreted as the QT interval adjusted to a standard heart rate of 60 beats per minute.

Genetic analysis.

Total genomic DNA was purified from whole-blood samples obtained from subjects using the PUREGENE DNA isolation kit (Gentra, Minneapolis, MN). DNA concentration was quantified using standardized fluorometric readings on a Hoefer DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech, San Francisco, CA). Each sample was diluted to a final concentration of 5 ng/μl.

Genotypes for rs10494366 and rs10918594 were determined using a Mass-ARRAY SNP Genotyping System (Sequenom, San Diego, CA) (21). This genotyping system uses single-base extension reactions to create allele-specific products that are separated and scored in a matrix-assisted laser desorption ionization/time of flight mass spectrometer. Primers for

PCR amplification and extension reactions were designed using the MassARRAY Assay Design Software (Sequenom).

Statistical analysis.

Maximum likelihood allele and genotype frequencies for each SNP were calculated from unrelated probands and were tested for departures from Hardy-Weinberg equilibrium using both χ^2 and exact tests. Estimates of linkage disequilibrium between SNPs were determined by calculating pairwise D' and r^2 statistics in unrelated individuals. As previously reported, the microsatellite markers from a 10-cM genome scan were used to examine and correct self-reported familial relationships (16). Association between each SNP and the phenotype under an additive genetic model was tested using variance components methods implemented in SOLAR (22). Tests of statistical significance for the additive genetic model were based on the likelihood ratio statistic incorporating the familial correlation structure. Despite the strong a priori nature of the hypothesis, we used two-sided tests of significance. All analyses were performed separately in European Americans and African Americans. Within each ethnic group, analyses were performed in all participants and in diabetic subjects only, excluding subjects using any QT-altering medication.

The following procedure was used to estimate the ethnicity-specific magnitude of the effect of each polymorphism while adjusting for the covariates. First, the residuals from the variance components model adjusting for familial relationships and containing the covariates but not the polymorphisms were computed. Second, using the above variance components model without the polymorphisms, the predicted value of a woman, diagnosed with diabetes, with the mean RR interval (European American = 0.0147, African American = 0.0144, European American with diabetes = 0.0145, and African American with diabetes = 0.0142), the mean age (European American = 61.5, African American = 58.6, European American with diabetes = 61.9, and African American with diabetes = 58.8 years), and mean BMI (European American = 31.8, African American = 33.8, European American with diabetes = 32.5, and African American with diabetes = 34.0 kg/m^2) was added to the residuals. Third, the genotypic means of the residuals scaled to the predicted value of the above “individual” were computed to give a sense of the observed differences between the three genotypes for the respective polymorphisms.

To estimate the ethnicity-specific genetic contribution to QT interval duration, a series of heritability analyses were computed using SOLAR (22). In these analyses, the overall phenotypic variation was partitioned into individual variance components due to polygenic effects (multiple unmeasured genes under an additive variance), covariates (e.g., RR interval, age, sex, diabetes affection status, and BMI), and random environmental effects. The estimated heritability (h^2) is defined as the ratio of the genetic variance component to the residual phenotypic variance and is an estimate of the familiality of the trait. To estimate the heritability and the proportion of variation explained by each polymorphism while adjusting for the above covariates, the heritability and proportion of variation in QT interval duration were computed with and without each individual polymorphism.

RESULTS

The clinical characteristics of the 624 European-American men and women in 331 families and 127 African-American men and women in 69 families are presented in Table 1. As expected, the Diabetes Heart Study participants carry a high burden of coronary artery disease risk factors with elevated BMI, rates of hypertension, and current and past smoking (Table 1).

DNA from these participants was genotyped for two SNPs (rs10494366 and rs10918594) selected a priori based on the original report of association with QT interval duration (11). For both SNPs, the genotyping success rate was > 90%, and the genotyping consensus rate for duplicate DNA samples within and across DNA plates was 100%. In the European-American individuals, genotype frequencies were consistent with those expected under Hardy-Weinberg equilibrium ($P > 0.01$). The two SNPs (minor allele frequency for G allele 0.35 and G allele 0.33, respectively) were in strong linkage disequilibrium (D' 0.85) and were correlated ($r^2 = 0.65$). In the African-American individuals, rs10494366 genotypes (major allele frequency for G allele 0.54) were consistent with Hardy-Weinberg expectations. However, rs10918594 (major allele frequency for G allele 0.55) did not conform to Hardy-Weinberg expectations because of a deficiency in the observed number of heterozygotes ($P = 0.001$), possibly due to admixture of chromosomes of African and European ancestry, in which substantial frequency differences exist. In African Americans, the two SNPs showed less linkage disequilibrium with each other ($D' = 0.31$) and substantially less correlation ($r^2 = 0.08$).

In European-American individuals not taking any medication known to alter QT interval ($n = 624$), the minor allele G at each locus was strongly associated with QT interval duration in an additive genetic model adjusting for RR interval, age, sex, diabetes affection status, and BMI (Table 2). There were 5.3- and 6.4-ms differences in adjusted QT interval duration for each additional minor allele for the two SNPs, respectively ($P = 5.7 \times 10^{-5}$, $P = 1.5 \times 10^{-6}$). Minor homozygotes of rs10494366 had a 9.3-ms difference in QT interval duration, and rs10918594 minor homozygotes had a 12.5-ms difference in a QT interval duration compared with major homozygotes. In analyses restricted to the diabetic subsample of 514 European-American men and women, there was a 5.9- and 7.1-ms difference in adjusted QT interval duration for each additional minor allele for the two SNPs, respectively ($P = 5.1 \times 10^{-5}$, $P = 1.6 \times 10^{-6}$). Minor homozygotes for the two SNPs had longer QT interval duration compared with major homozygotes (11.3- and 13.9-ms difference, respectively). Statistical significance was retained for both SNPs in the European-American and European-American diabetic individuals when analysis of the heart rate–corrected QTc was performed (additive model P values of 0.02 and 0.01 for rs10494366 and rs10918594, respectively; data not shown). When individuals with atrial fibrillation or bundle branch blocks were excluded, there were no appreciable differences in the point estimates or statistical significance (additive model P values of 1.2×10^{-4} and 1.3×10^{-5} in European-American individuals; additive model P values of 9.6×10^{-5} and 9.9×10^{-6} in European-American diabetic subjects). In addition, similar effects were observed when analyses were performed on the excluded sample of European-American individuals known to be taking a QT-prolonging medication ($P < 0.05$; data not shown).

In the entire European-American sample, rs10494366 and rs10918594 explained 1.2 and 2.0% of the variation in QT interval duration, and in the diabetic European-American sample, these SNPs explained 1.5 and 2.2% of the variation in QT interval duration, respectively (Table 3). The heritability of QT interval duration adjusted for RR interval, age, sex, diabetes affection status, and BMI was 0.476 ± 0.14 in the total sample and 0.323 ± 0.18 in the diabetic subsample. A formal test of interaction of diabetes affection and SNP-QT interval effect in the European American sample was not significant ($P = 0.252$ for rs10494366; $P = 0.345$ for rs10918594) but was underpowered because of the small number of individuals free of overt diabetes.

In African-American individuals not taking any QT-altering medications, there was no evidence of association between rs10494366 and QT interval duration in the total sample ($n = 127$; $P = 0.24$) or in the diabetic subsample ($n = 115$; $P = 0.14$). However, rs10918594 exhibited a trend toward association with QT interval in both the total African-American sample (two-sided $P = 0.08$; one-sided $P = 0.04$) and the diabetic African-American subsample (two-sided $P = 0.04$; one-sided $P = 0.02$). The results for rs10918594 should be interpreted with caution, however, because this SNP did not conform to Hardy-Weinberg expectations in African-American individuals. In the African-American sample, the G allele for each polymorphism is the major allele, but the observed effect is in the same direction as in the European-American sample with GG homozygotes having a longer QT interval duration than the alternate homozygotes. Power in the African-American sample was limited to detect an effect of *NOS1AP* SNPs due to small sample size. Specifically, under an additive model, the African-American sample provides ~0.80 power at $\alpha = 0.05$ to detect a difference of 7.4 ms in QT interval per major allele copy; here, the difference of 7.4 ms is obtained by multiplying the approximate within-genotype SD for QT interval in African Americans of ~20 ms by the proportion of SD detectable of 0.37 (i.e., 20 × 0.37 = 7.4) (Supplemental Table, available in the online appendix at http://dx.doi.org/10.2337/db07-1365).

DISCUSSION

These data strongly replicate the findings from three previous studies that common noncoding variants at the *NOS1AP* locus are associated with QT interval duration (11–13). The two SNPs evaluated—rs10918594, located in the promoter region upstream of *NOS1AP*, and rs10494366, located in intron 1 of *NOS1AP*—are separated by 55 Kb genomic DNA, have no known biological function, and are not highly correlated with any known functional polymorphisms. Taken together, these observations suggest that a causal untyped variant exists that is highly correlated with rs10918594 and rs10494366.

The data reported in the current study extend the prior findings to a cohort of families aggregating type 2 diabetes, which are known to be at increased risk for disordered myocardial repolarization and increased risk of ventricular arrhythmias (23,24). In diabetic patients, the magnitude of the estimated effect (β) of *NOS1AP* SNPs on QT interval duration was larger than that observed in the total sample of diabetic and nondiabetic subjects or in the prior reports of community-based samples (11–13). Although the formal test of interaction of diabetes affection and SNP-QT interval effect was not significant ($P = 0.25$

for rs10494366, $P = 0.35$ for rs10918594 in European-American individuals), the test was underpowered due to the small number of nondiabetic individuals in the study population. It should be noted that the nondiabetic subjects were also relatively obese, with many of these individuals having hypertension and metabolic syndrome (many were likely pre-diabetic). However, when comparing the estimated effect sizes observed in our European-American diabetic subjects to those in the subjects of the community-based Rotterdam Study and the Old Order Amish, our estimates are statistically larger (rs10494366 European-American diabetic subjects β = 5.85, Rotterdam Study β = 3.8, *P* value = 0.15; rs10494366 European-American diabetic subjects β = 5.85, Old Order Amish β = 2.60, *P* value = 0.02; and rs10918594 European-American diabetic subjects β = 7.07, Rotterdam Study β = 3.6, *P* value = 0.02). In addition, a test comparing diabetic and nondiabetic individuals suggests that there is a significant difference in QT interval duration between the two groups (two-sided *P* values of 0.029 and 0.088 for rs10494366 and rs10918594, respectively) in our study population. We observed modest association of one *NOS1AP* variant in the African-American sample, in whom the minor QT-prolonging allele in European Americans is the major allele, but power was limited to detect an effect because of the small sample size. Notably, the variant modestly associated with QT interval in the African Americans did not conform to Hardy-Weinberg expectations because of a deficiency in the observed number of heterozygotes compared with the expected number. However, because the genotyping success rate was > 91.6% and the genotyping consensus rate for duplicate DNA samples within and across DNA plates was 100%, it is unlikely that this deviation from Hardy-Weinberg expectations is due to genotyping error. For variants such as these *NOS1AP* SNPs with substantial frequency differences between chromosomes of African and European ancestry, deviation from Hardy-Weinberg expectations due to a deficiency of heterozygotes can be observed simply because of admixture of chromosomes of different ancestry, as is found in the African Americans (25).

Genetic association studies have been plagued by lack of reproducibility of reported associated variants (26). In large part, inappropriately permissive thresholds for declaring statistical significance and samples underpowered to detect modest effects underlie the previously chaotic field of genetic associations. The problem was previously more modest in scope because genotyping platforms limited association tests to a handful of variants in candidate genes that have higher prior probabilities of harboring functional alleles. In the era of genome-wide association studies, the problem is compounded by the availability of hundreds of thousands of tests of variants with generally lower prior probability for association with a trait. Use of appropriately rigorous statistical thresholds and replication in multiple independent samples is critical to assure that apparent associations are true-positive results. Thus, our finding that the previously reported association of common variants in *NOS1AP* with QT interval duration is strongly replicated is an important validation of the original report.

Diabetes is a potent risk factor for SCD (8,9,27). QT interval prolongation is known to be associated with both type 1 and type 2 diabetes (23,24,28–33). QT interval prolongation is also known to predict increased risk of SCD and coronary heart disease death in the general population (5) and is a particularly potent risk factor in the diabetic population (23). Whether the relationship of diabetes to QT prolongation is through acquired autonomic

dysfunction (34), which presumably might have a nongenetic basis or reflects some fundamental interaction with genetic factors of the diabetic host, is unknown. However, the demonstration that genetic variants in *NOS1AP* reproducibly alter QT interval duration in families aggregating diabetes demonstrates that genetic effects are not swamped out by the strong effect of diabetes on repolarization. Rather, the opposite appears to be true: the reduced repolarization reserve of diabetes (35,36) shows a synergistic interaction with genetic variants that alter myocardial repolarization. It is thus notable that the strength of the effect of *NOS1AP* SNPs in European-American diabetic subjects in the current report, 11.3- to 13.9-ms differences between alternate homozygotes, is much greater than that observed in the previous reports by Arking et al. (11) (4–8 ms) Aarnoudse et al. (12), (6.3–7.2 ms) and Post et al. (13) (0.2–6.1 ms). Of additional interest, 34% (n = 264) of all European-American diabetic subjects (n = 778) in the Diabetes Heart Study is using a QT-altering medication (data not shown). It is therefore possible that this medication use in the diabetic population, in concert with the presence of QT-prolonging alleles of *NOS1AP*, may increase the risk for arrhythmias and SCD in this patient subset. This is an important issue to address in the future.

Strengths of our study include the well-phenotyped study sample, the precise electrocardiographic measures, the ability to adjust for multiple potential confounders, and the modest heritability of QT interval in the study population. In fact, our heritability results in European Americans (h^2 = 0.48) are notably higher than the estimates in the Framingham Heart Study (h^2 0.35) (10) or in female (h^2 = 0.25) (37) or male (h^2 = 0.36) (38) twins. In addition, the availability of detailed medication information allowed exclusion of individuals on QT-altering medications. Some limitations apply. Our study had better power to detect *NOS1AP* effects on QT interval duration in individuals of European ancestry but was clearly underpowered to demonstrate convincingly whether *NOS1AP* variants influence QT interval duration in individuals of African ancestry. Ultimately, testing in large numbers of African Americans will be required to establish whether common genetic variants at *NOS1AP* are related to QT interval duration. Differences in the genetic architecture of QT interval duration or in patterns of linkage disequilibrium between individuals of European and African ancestry could contribute to lack of association in African Americans. The small number of SCDs and difficulty in its ascertainment precluded an analysis of the influence of *NOS1AP* variants on SCD risk.

In summary, these data provide convincing replication of the association with continuous QT interval duration of *NOS1AP* variants in families of European ancestry aggregating diabetes. Further studies in diabetic individuals will be needed to test the hypothesis raised here that genetic variants influencing QT interval duration are particularly important with regard to the burden of SCD among diabetic subjects. Genetic studies of QT interval variation in diabetic individuals, who have reduced repolarization reserve, may be particularly fruitful in identifying variants contributing to risk of arrhythmia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

ECG	electrocardiogram
NOS1AP	nitric oxide synthase 1 adaptor protein
QTc	corrected QT interval
SCD	sudden cardiac death
SNP	single nucleotide polymorphism

REFERENCES

1. Heart Disease and Stroke Statistics: 2005 Update. Dallas, TX, American Heart Association, 2005
2. Schouten EG, Dekker JM, Meppelink P, Kok FJ, Vandenbroucke JP, PoolJ: QT interval prolongation predicts cardiovascular mortality in an apparently healthy population. Circulation 84:1516–1523, 1991 [PubMed: 1914093]
3. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J: QTc prolongation measured by standard 12-lead electrocardiography is an independent risk factor for sudden death due to cardiac arrest. Circulation 83:1888–1894, 1991 [PubMed: 2040041]
4. Siscovick DS, Raghunathan TE, Rautaharju P, Psaty BM, Cobb LA, Wagner EH: Clinically silent electrocardiographic abnormalities and risk of primary cardiac arrest among hypertensive patients. Circulation 94:1329–1333, 1996 [PubMed: 8822988]
5. Straus SM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, Deckers JW, Kingma JH, Sturkenboom MC, Stricker BH, Witteman JC: Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. J Am Coll Cardiol 47:362–367, 2006 [PubMed: 16412861]
6. Roden DM: Drug-induced prolongation of the QT interval. N Engl J Med 350:1013–1022, 2004 [PubMed: 14999113]
7. Friedlander Y, Siscovick DS, Weinmann S, Austin MA, Psaty BM, LemaitreRN, Arbogast P, Raghunathan TE, Cobb LA: Family history as a risk factor for primary cardiac arrest. Circulation 97:155–160, 1998 [PubMed: 9445167]
8. Jouven X, Desnos M, Guerot C, Ducimetiere P: Predicting sudden death inthe population: the Paris Prospective Study I. Circulation 99:1978–1983, 1999 [PubMed: 10209001]
9. Friedlander Y, Siscovick DS, Arbogast P, Psaty BM, Weinmann S, LemaitreRN, Raghunathan TE, Cobb LA: Sudden death and myocardial infarction in first degree relatives as predictors of primary cardiac arrest. Atherosclerosis 162:211–216, 2002 [PubMed: 11947916]
10. Newton-Cheh C, Larson MG, Corey DC, Benjamin EJ, Herbert AG, Levy D,D'Agostino RB, O'Donnell CJ: QT interval is a heritable quantitative trait with evidence of linkage to chromosome 3 in a genome-wide linkage analysis: The Framingham Heart Study. Heart Rhythm 2:277–284, 2005 [PubMed: 15851319]

11. Arking DE, Pfeufer A, Post W, Kao WH, Newton-Cheh C, Ikeda M, West K,Kashuk C, Akyol M, Perz S, Jalilzadeh S, Illig T, Gieger C, Guo CY, Larson MG, Wichmann HE, Marban E, O'Donnell CJ, Hirschhorn JN, Kaab S, Spooner PM, Meitinger T, Chakravarti A: A common genetic variant in the NOS1 regulator NOS1AP modulates cardiac repolarization. Nat Genet 38:644–651, 2006 [PubMed: 16648850]
12. Aarnoudse AJ, Newton-Cheh C, de Bakker PI, Straus SM, Kors JA, HofmanA, Uitterlinden AG, Witteman JC, Stricker BH: Common NOS1AP variants are associated with a prolonged QTc interval in the Rotterdam Study. Circulation 116:10–16, 2007 [PubMed: 17576865]
13. Post W, Shen H, Damcott C, Arking DE, Kao WH, Sack PA, Ryan KA, Chakravarti A, Mitchell BD, Shuldiner AR: Associations between genetic variants in the NOS1AP (CAPON) gene and cardiac repolarization in the Old Order Amish. Hum Hered 64:214–219, 2007 [PubMed: 17565224]
14. Frazer KA, Ballinger DG, Cox DR, et al. : A second generation human haplotype map of over 3.1 million SNPs. Nature 449:851–861, 2007 [PubMed: 17943122]
15. Wagenknecht LE, Bowden DW, Carr JJ, Langefeld CD, Freedman BI, RichSS: Familial aggregation of coronary artery calcium in families with type 2 diabetes. Diabetes 50:861–866, 2001 [PubMed: 11289053]
16. Bowden DW, Rudock M, Ziegler J, Lehtinen AB, Xu J, Wagenknecht LE, Herrington D, Rich SS, Freedman BI, Carr JJ, Langefeld CD: Coincident linkage of type 2 diabetes, metabolic syndrome, and measures of cardiovascular disease in a genome scan of the Diabetes Heart Study. Diabetes 55:1985–1994, 2006 [PubMed: 16804067]
17. Lange LA, Bowden DW, Langefeld CD, Wagenknecht LE, Carr JJ, Rich SS,Riley WA, Freedman BI: Heritability of carotid artery intima-medial thickness in type 2 diabetes. Stroke 33:1876–1881, 2002 [PubMed: 12105369]
18. Prineas RJ, Crow RS, Blackburn H: The Minnesota Code: Manual of Electrocardiographic Findings. Boston, John Wright, 1982
19. Marquette 12SL ECG Analysis Program: Physician's Guide, Revision B. Waukesha, Wisconsin, GE Healthcare, 2006, p. 3–6
20. Bazett HC: An analysis of time relations of the electrocardiogram. Heart 7:353–370, 1920
21. Oeth P, Beaulieu M, Park C, Kosman D, del Mistro G, van den Boom D,Jurinke C: iPLEX assay: increased plexing efficiency and flexibility for MassARRAY system through single base primer extension with mass-modified terminators [article online], 2005. Sequenom Application Note. Available from http://www.sequenom.com/Genetic-Analysis/Applications/iPLEX-Genotyping/Literature. Accessed 4 May 2007
22. Almasy L, Blangero J: Multipoint quantitative-trait linkage analysis ingeneral pedigrees. Am J Hum Genet 62:1198–1211, 1998 [PubMed: 9545414]
23. Whitsel EA, Boyko EJ, Rautaharju PM, Raghunathan TE, Lin D, Pearce RM, Weinmann SA, Siscovick DS: Electrocardiographic QT interval prolongation and risk of primary cardiac arrest in diabetic patients. Diabetes Care 28:2045–2047, 2005 [PubMed: 16043757]
24. Cardoso CR, Salles GF, Deccache W: Prognostic value of QT interval parameters in type 2 diabetes mellitus: results of a long-term follow-up prospective study. J Diabetes Complications 17:169–178, 2003 [PubMed: 12810239]
25. Hartl DL, Clark AG: Principles of Population Genetics. Sunderland, MA, Sinauer Associates, 2006
26. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K: A comprehensive review of genetic association studies. Genet Med 4:45–61, 2002 [PubMed: 11882781]
27. Solomon SD, Zelenkofske S, McMurray JJ, Finn PV, Velazquez E, Ertl G, Harsanyi A, Rouleau JL, Maggioni A, Kober L, White H, Van de Werf F, Pieper K, Califf RM, Pfeffer MA: Sudden death in patients with myocardial infarction and left ventricular dysfunction, heart failure, or both. N Engl J Med 352:2581–2588, 2005 [PubMed: 15972864]
28. Zhang Y, Xiao J, Wang H, Luo X, Wang J, Villeneuve LR, Zhang H, Bai Y, Yang B, Wang Z: Restoring depressed HERG K channel function as a mechanism for insulin treatment of abnormal QT prolongation and associated arrhythmias in diabetic rabbits. Am J Physiol Heart Circ Physiol 291:H1446–H1455, 2006 [PubMed: 16617123]

29. Robinson RT, Harris ND, Ireland RH, Lee S, Newman C, Heller SR: Mechanisms of abnormal cardiac repolarization during insulin-induced hypoglycemia. Diabetes 52:1469–1474, 2003 [PubMed: 12765959]

30. Rutter MK, Viswanath S, McComb JM, Kesteven P, Marshall SM: QT prolongation in patients with type 2 diabetes and microalbuminuria. Clin Auton Res 12:366–372, 2002 [PubMed: 12420081]

31. Veglio M, Bruno G, Borra M, Macchia G, Bargero G, D'Errico N, Pagano GF, Cavallo-Perin P: Prevalence of increased QT interval duration and dispersion in type 2 diabetic patients and its relationship with coronary heart disease: a population-based cohort. J Intern Med 251:317–324, 2002 [PubMed: 11952882]

32. Christensen PK, Gall MA, Major-Pedersen A, Sato A, Rossing P, Breum L, Pietersen A, Kastrup J, Parving HH: QTc interval length and QT dispersion as predictors of mortality in patients with non-insulin-dependent diabetes. Scand J Clin Lab Invest 60:323–332, 2000 [PubMed: 10943602]

33. Sawicki PT, Kiwitt S, Bender R, Berger M: The value of QT interval dispersion for identification of total mortality risk in non-insulin-dependent diabetes mellitus. J Intern Med 243:49–56, 1998 [PubMed: 9487331]

34. Wei K, Dorian P, Newman D, Langer A: Association between QT dispersion and autonomic dysfunction in patients with diabetes mellitus. J Am Coll Cardiol 26:859–863, 1995 [PubMed: 7560609]

35. Roden DM: Taking the "idio" out of "idiosyncratic": predicting torsades de pointes. Pacing Clin Electrophysiol 21:1029–1034, 1998 [PubMed: 9604234]

36. Lengyel C, Virag L, Biro T, Jost N, Magyar J, Biliczki P, Kocsis E, Skoumal R, Nanasi PP, Toth M, Kecskemeti V, Papp JG, Varro A: Diabetes mellitus attenuates the repolarization reserve in mammalian heart. Cardiovasc Res 73:512–520, 2007 [PubMed: 17182020]

37. Carter N, Snieder H, Jeffery S, Saumarez R, Varma C, Antoniades L, Spector TD: QT interval in twins. J Hum Hypertens 14:389–390, 2000 [PubMed: 10878701]

38. Russell MW, Law I, Sholinsky P, Fabsitz RR: Heritability of ECG measurements in adult male twins. J Electrocardiol 30 (Suppl.):64–68, 1998 [PubMed: 9535482]

TABLE 1

Clinical characteristics of 624 European-American and 127 African-American Diabetes Heart Study participants after exclusion of individuals with history of myocardial infarction or on QT-altering medications

	European-American men	European-American women	African-American men	African-American women
n	333	291	50	77
Age (years)	62.2 ± 9.4	60.9 ± 10.4	59.1 ± 8.5	59.6 ± 9.7
BMI (kg/m^2)	30.6 ± 5.3	32.5 ± 7.4	31.4 ± 6.3	34.5 ± 7.3
Total cholesterol (mg/dl)	178.2 ± 38.6	198.9 ± 44.3	178.9 ± 33.0	194.5 ± 35.7
Smoking				
Current	17.2 (57)	17.6 (51)	42.0 (21)	13.3 (10)
Past	59.0 (197)	23.8 (69)	46.0 (23)	33.3 (26)
Never	23.8 (79)	58.6 (171)	12.0 (6)	53.3 (41)
Diabetes diagnosis	85.3 (284)	79.0 (230)	96.0 (48)	87.0 (67)
Duration of diabetes (years)	10.6 ± 7.6	9.5 ± 6.1	11.0 ± 8.2	11.2 ± 8.3
Hypertension	79.9 (266)	83.2 (242)	78.0 (39)	87.0 (67)
RR interval	0.015 ± 0.0027	0.0143 0.0024	0.0151 ± 0.0026	0.0143 ± 0.0023
Unadjusted QT interval duration (ms)	394.0 ± 34.2	392.3 ± 32.1	398.0 ± 34.7	392.8 ± 30.0
QRS duration (ms)	94.3 ± 10.6	87.5 ± 9.7	93.9 ± 12.7	85.7 ± 8.8

Data are means ± SD.

TABLE 2

Association analysis of two *NOS1AP* SNPs and QT interval in European-American and African-American Diabetes Heart Study participants free of myocardial infarction or QT-altering medication use

SNP[†]	Alleles (major/ minor)	Frequency of G allele	Genotypic means (QT interval in ms)			ß for additive genetic model	*P* value for additive genetic model[*]
All European Americans							
rs10494366	T/G	0.35	TT (*n* = 238) 386.4 ± 20.2	TG (*n* = 266) 392.8 ± 19.1	GG (*n* = 64) 395.7 ± 21.7	5.3 ± 1.3	**5.7 × 10 $^{-5}$**
rsl0918594	C/G	0.33	CC (*n* = 254) 386.9 ± 19.1	CG (*n* = 239) 393.9 ± 20.1	GG (*n* = 58) 399.4 ± 21.2	6.4 ± 1.3	**1.5 × l0 $^{-6}$**
Diabetic European Americans only							
rs10494366	T/G	0.35	TT (*n* = 203) 385.2 ± 20.9	TG (*n* = 214) 391.8 ± 19.2	GG (*n* = 54) 396.5 ± 22.2	5.9 ± 1.4	**5.1 × 10 $^{-5}$**
rsl0918594	C/G	0.33	CC (*n* = 213) 385.7 ± 19.5	CG (*n* = 195) 392.9 ± 20.1	GG (*n* = 47) 399.6 ± 22.7	7.1 ± 1.5	**1.6 × l0 $^{-6}$**
All African Americans							
rs10494366	G/T	0.54	TT (*n* = 22) 386.1 ± 20.6	TG (*n* = 51) 385.1 ± 18.2	GG (*n* = 40) 391.5 ± 23.4	3.2 ± 2.7	0.24
rsl0918594	G/C	0.55	CC (*n* = 36) 387.7 ± 17.8	CG (*n* = 36) 385.1 ± 18.6	GG (*n* = 41) 395.7 ± 23.4	4.1 ± 2.4	0.08
Diabetic African Americans only							
rs10494366	G/T	0.54	TT (*n* = 19) 383.3 ± 20.4	TG (*n* = 50) 382.1 ± 17.7	GG (*n* = 34) 390.7 ± 24.8	4.4 ± 2.9	0.14
rsl0918594	G/C	0.55	CC (*n* = 32) 385. 8 ± 17 8	CG (*n* = 33) 382. 7 ± 18 4	GG (*n* = 38) 395. 8 ± 23 4	5.2 ± 2.5	**0.04**

[*] *P* values reported are for the additive genetic model for association with QT interval adjusted for RR interval, age, sex, diabetes affection status (where appropriate), and BMI. *P* values shown in bold represent significance at the $P < 0.05$ level.

[†] Chromosome 1 physical positions: 160352309 (rs10494366) and 160297312 (rs10918594) relative to National Center for Biotechnology Information dbSNP build 126.

TABLE 3

Proportion of variance in QT interval duration explained by covariates and estimates of heritability in the European-American sample with and without each SNP individually under an additive genetic model

Covariates	Proportion of variance attributable to covariates	Proportion of variance attributable to each SNP	h^2_r
All European Americans			
RR interval, age, sex, diabetes, and BMI	0.631		0.476 ± 0.14
Covariates plus rs10494366	0.643	0.012	0.468 ± 0.14
Covariates plus rs10918594	0.651	0.020	0.418 ± 0.14
Diabetic European Americans only			
RR interval, age, sex, and BMI	0.624		0.323 ± 0.18
Covariates plus rs10494366	0.639	0.0152	0.294 ± 0.18
Covariates plus rs10918594	0.646	0.022	0.266 ± 0.17

h^2_r data are means SE. h^2_r represents the residual heritibility after adjusting for covariates and each SNP individually.