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Genome-wide association study of electrocardiographic conduction measures in an isolated founder population: Kosrae

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Abstract

Background—Cardiac conduction, as assessed by electrocardiographic PR interval and QRS duration, is an important electrophysiological trait and a determinant of arrhythmia risk.

Objective—We sought to identify common genetic determinants of these measures of cardiac conduction time.

Methods—We examined 1604 individuals from the island of Kosrae, Federated States of Micronesia, an isolated founder population. We adjusted for covariates and estimated the heritability of quantitative electrocardiographic QRS duration, PR interval and secondarily its subcomponents P wave duration and PR segment. Finally, we performed a genome-wide association study (GWAS) in a subset of 1262 individuals genotyped using the Affymetrix GeneChip Human Mapping 500K microarray.

Results—The heritability of PR interval was 34% (SE 5%, $p=4\times10^{-18}$), of PR segment 31% (SE 6%, $p=3.2\times10^{-13}$) and P wave duration 17% (SE 5%, $p=5.8\times10^{-6}$) but for QRS duration only 3% (SE 4%, p=0.20). Hence, GWAS was performed only for PR interval and its subcomponents. A total of 338,049 SNPs passed quality criteria. For PR interval, the most significantly associated SNPs were located in and downstream of the alpha-subunit of the cardiac voltage-gated sodium channel gene *SCN5A* with a 4.8 msec (SE 1.0) or 0.23 standard deviation increase in adjusted PR interval for each minor allele copy of rs7638909 ($p=1.6\times10^{-6}$, minor allele frequency 0.40). These SNPs were also associated with P wave duration ($p=1.5\times10^{-4}$) and PR segment (p=0.01) but not with QRS duration ($p\geq0.22$).

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Conflicts of interest: None

Conclusions—PR interval and its subcomponents showed substantial heritability in a South Pacific islander population and were associated with common genetic variation in *SCN5A*.

Keywords

Conduction; electrocardiography; electrophysiology; genetics; ion channels

Introduction

Electrocardiographic PR interval and QRS duration are measures of cardiac conduction time. PR interval duration reflects conduction of the electrical signal from the SA-node through the conduction system to the ventricular myocardium but primarily through the AV-node. It can be subdivided into P wave duration and the PR segment, from the end of the P wave to the beginning of the QRS. The QRS complex reflects conduction through the ventricular myocardium. Experimental and clinical data suggest a role for global atrial conduction slowing in the pathogenesis of atrial arrhythmias[1] and QRS prolongation is associated with ventricular arrhythmias and sudden cardiac death.[2]

The PR interval has evidence of a heritable component with most previous heritability estimates in the range of 0.34–0.46, which could result from rare or common genetic variants or both.[3–5] Rare coding[6,7] and common noncoding[8] variants in the alpha-subunit of the cardiac voltage-gated sodium channel gene, *SCN5A*, have been reported to cause delayed conduction and PR interval prolongation but to date most of the PR interval heritability remains unexplained. PR interval has also been shown to be influenced by other factors including sex, age, heart rate and dromotropic drugs, such as those acting on the cardiac sodium channel. QRS duration, on the other hand, has not shown evidence of significant heritability in previous studies.[5,9]

A genome-wide association study (GWAS) can be used to identify genes (known or unknown) and variants in them that are associated with cardiac conduction phenotypes. GWAS uses dense maps of common genetic single nucleotide polymorphisms (SNPs) to examine whether genotype is significantly associated with differences in phenotype. The GWAS approach has only recently been made possible by advances in genotyping and genomic mapping and has successfully identified genes involved in a number of common, multifactorial diseases including atrial fibrillation[10] as well as genetic determinants of quantitative traits such as electrocardiographic QT interval[11]. In aggregate, such studies have confirmed the common disease-common variant hypothesis, that common genetic variants influence heritability of common diseases and observed variation in quantitative traits. Many of the genes and pathways identified in this systematic manner, such as *NOS1AP* in QT interval,[11] had not previously been linked to the trait of interest and thus provide novel physiological information.

We hypothesized that a systematic search for common genetic variants with an influence on PR interval, and secondarily its subcomponents, and QRS duration could identify novel molecular mechanisms involved in cardiac conduction which would then be potential candidates for arrhythmogenesis. We performed a GWAS in a community-based sample from the island of Kosrae, Federated States of Micronesia, an isolated founder population. This population exhibits long-range linkage disequilibrium,[12] potentially making association scanning with high genomic coverage feasible.

Methods

Study sample

Inclusion of individuals and variables examined in the Kosrae study has been described previously.[13] Briefly, residents of Kosrae were examined at three different time points: in 1994, 2001 and 2003. We studied the sample examined in 2001 which included a total of 2170 individuals.[14] All individuals were examined with recording of anthropometric traits, blood pressure, several blood biomarkers, medications (the island has a limited formulary), self-reported ethnicity, and lifestyle habits. Self-reported familial relationships were investigated by a genetics counsellor working on Kosrae who was able to construct a family tree that included most of the Islanders and which was later and revised based on identity-by-descent estimates from SNP data using PLINK.[15] One individual was excluded for self report of non-Micronesian ancestry. For the three pairs of monozygotic twins identified, the average trait value was used in analyses. After exclusions, there were 1604 individuals in the PR interval heritability analysis and 1588 individuals in the QRS duration heritability analysis. PR interval GWAS was completed in the 1262 individuals free of exclusions with available genome-wide genotypes passing quality control. In a secondary analysis after our initial PR interval GWAS results were obtained, additional ECG measurements were made in the subset of individuals free of exclusions with genotypes in the GWAS and included 1133 individuals with P wave duration and 1131 individuals with PR segment measures. Informed consent in Kosraen was obtained from all subjects. The study protocol was approved by the Institutional Review Boards of Rockefeller University, Massachusetts General Hospital and the Massachusetts Institute of Technology. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Electrocardiographic trait measurement

Twelve-lead electrocardiograms were recorded at a paper speed of 50 mm per second in 1756 individuals using a Universal ECG (QRS Diagnostic LLC, Plymouth, MN). De-identified paper electrocardiograms were scanned into digital images at high resolution. Quantitative measures of QRS duration, PR interval, P wave duration and PR segment were obtained using digital calipers in Rigel 1.7.3 (AMPS LLC, New York, NY) from two cardiac cycles of lead II and one cycle each from V2 and V5. PR interval was measured from the onset of the P wave to the onset of the QRS complex and PR segment from the offset of the P wave to the onset of the QRS complex. Measurements in all analyzed individuals were made at two time points with high agreement for mean PR interval (r=0.90) but lower for mean QRS duration (r = 0.60).

Genotyping and Quality Control

Genotyping was performed using the Affymetrix GeneChip Human Mapping 500K microarray at Affymetrix (San Francisco, CA). Genotypes were called using the Bayesian Robust Linear Modeling using Mahalanobis Distance (BRLMM) algorithm.[16] SNPs were excluded for the following reasons: genotype call rate below 95%, twelve or more Mendelian errors, minor allele frequency below 0.01or mapping to more than one locus in the human genome. A total of 338,049 SNPs passed quality criteria on the 22 autosomes.

Phenotype modeling

We excluded from analyses of PR interval and its subcomponents individuals with Wolf-Parkinson-White pattern (n=1), those using drugs that slow AV-nodal conduction (n=4), those with atrial fibrillation (n=1). No individuals examined for this study had a pacemaker. In QRS analyses, we excluded individuals with mean QRS duration >120 ms or right bundle branch (n=36). Normality of traits was confirmed.

We adjusted for covariates using multivariable linear regression models. To create regression models, we first examined univariable regression models for relevant covariates. The following covariates were significant ($p<0.05$) in univariable linear regression models: age, sex, BMI, height, weight, waist, waist-to-hip ratio, body surface area, lean body mass, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, pulse pressure, hypertension, heart rate, total cholesterol, LDL, HDL, triglycerides, creatinine, diabetes, fasting blood sugar, and HbA1c.

Covariates with $p<0.05$ on univariable analysis were included in stepwise multiple regression models for each measure in cardiac cycle-specific and averaged trait values (from up to four measures). The variability explained by each model (as estimated by the coefficient of determination, r^2) was compared between the cycle-specific and mean trait models, under the assumption that more precise measures would have a greater proportion of variation explainable by clinical covariates. For all traits the r^2 in regression models and the correlation of two independent sets of measures was greatest for the mean of all four measures which was therefore used for heritability estimation and association. Residuals from fully-adjusted models were standardized to mean 0, standard deviation 1 and served as the trait for subsequent heritability and genotype-phenotype association analyses. Phenotype modeling was performed in SPSS (SPSS v11.5.0, SPSS Inc, Chicago, Illinois).

Heritability

Additive heritability was estimated using variance components analysis as implemented in the Sequential Oligogenic Linkage Analysis Routines software (SOLAR v4.0.7, Southwest Foundation for Biomedical Research).

Genome-wide association analysis

The association strategy was designed to account for the small effective population size and the high degree of relatedness that exists on Kosrae due to the founder effect. Multiple strategies were examined and compared for type I and type II error rates in simulation experiments.[14] The final strategy tested for association within sibships drawn from the extended Kosrae pedigree, and combines scores from both within- and between-sibship association testing as implemented in the QFAM-Total procedure in PLINK.[15] An additive genetic model was tested in all analyses. We included a number of singletons ("sibships" of one) with low relatedness to the rest of the sample as defined by identity-by-descent (IBD) estimates ≤ 0.125 (equivalent to a first cousin relationship or less). We adjusted for test statistic inflation induced by relatedness between sibships by calculation of the genomic inflation factor and subsequent genomic control. Our analytic strategy thus attempts to maximize power by analyzing within and between components of association, while controlling the inflation in test statistics resulting from background relatedness on the island.

A quantile-quantile plot of the observed compared to expected $-\log_{10}$(p-value) was generated in R v2.6.0 (http://www.r-project.org) and the $-\log^{10}$ (p-value) plotted using Haploview v4.0.[17] Plots of correlation patterns for selected loci were generated in Haploview using pairwise r^2 of SNPs from 133 individuals drawn from the entire population who exhibit the lowest pairwise relatedness (pairwise IBD estimates ≤ 0.08).

Results

Phenotype modeling

For clinical characteristics of the study sample see Table 1. The final regression model for PR interval included age, sex, heart rate, weight and fasting blood sugar and the final model for QRS duration included sex, heart rate, body surface area and pulse pressure. For P wave and

PR segment the same covariates as for PR duration were used. The proportion of variation in phenotype explained by covariates (r^2) was modest: 17%, 6%, 5% and 10% respectively.

Heritability

The estimated heritability of PR interval was 0.34 (SE 0.05, $p=4\times10^{-18}$), suggesting that additive genetic factors explain 34% of variability in PR interval. The heritability of P wave duration was 0.17 (SE 0.053, $p=5.8\times10^{-6}$), and of PR segment was 0.31 (SE 0.057, $p=3.2\times10^{-13}$) while that of QRS duration was not significant (0.03, SE 0.04, p=0.20). The correlation to PR interval of its subcomponents was substantial (P wave r=0.48, PR segment r=0.86). Because of the significant heritability of PR interval and its subcomponents and the low heritability of QRS duration, genome-wide association testing was performed for PR interval and its subcomponents only.

Genome-Wide Association Study

A GWAS of PR interval was performed using 1262 individuals from 168 sibships and 189 singletons and the 338,049 SNPs that passed quality control. Mild inflation of test statistics was observed (Figure 1) with a genomic inflation factor λ_{GC} of 1.16. After genomic control to correct for relatedness between sibships, there was a slight excess of low p-values (high −log (p-value)). The ten results with the lowest p-values were all for non-coding SNPs at chromosome 3p22.2 (Figure 2) at the 3′ end of the alpha-subunit of the cardiac sodium channel gene, *SCN5A* (MIM 600163) and downstream (Figure 3, Supplementary Table 1). The top SNP result was for rs7638909 in intron 27 of *SCN5A* (minor allele frequency 0.40), which was associated with an effect size of 4.8 ms (SE 1.0) or 0.23 SD increase in PR duration for each additional minor allele copy ($p=1.6\times10^{-6}$, Table 2), explaining 2.0% of variation in PR interval. The second best result was for SNP rs2070488 (minor allele frequency 0.33), which was associated with a 5.0 ms (SE 1.1) or 0.24 SD decrease in PR interval per minor allele ($p=3.8\times10^{-6}$). The majority of the SNPs with the lowest p-values in the region are highly correlated and fall in one large block of strong linkage disequilibrium (Figure 4A). We tested for association of the top two SNPs in a multivariable linear regression model and found both to be significant (rs7638909, p=0.001; rs2070488, p=0.04), suggesting possible independent effects. However, examination of the haplotypes across the locus suggest that the opposite direction of effect for the top two SNPs may be explained by the fact that the minor allele of rs7638909 is located on a complementary haplotype containing the major allele of rs2070488 (Figure 4 B, C). As can be seen in Table 2, only one other SNP than the ten SNPs at *SCN5A* reached a p-value below 10^{-5}, rs2461751 (4.5 ms/minor allele, SE 1.0, MAF 0.44, $p=8.0\times10^{-6}$), on chromosome 2 and more than 200kb from any known gene.

GWAS for P wave duration and PR segment were performed in 1133 and 1131 individuals and genomic control performed for inflation factors of 1.34 and 1.27, respectively. For PR segment the most significant SNP, rs2008242, is located near MSX1 ($p=3\times10^{-6}$) and for P wave duration we note that a SNP, rs10925451, in the cardiac ryanodine receptor (RYR2) ranks among the top ten SNPs ($p=2.9\times10^{-5}$) (Supplementary table 2).

SCN5A in P wave duration, PR segment and QRS duration

Since the heritability of QRS duration was nonsignificant, we did not perform a genome-wide association scan for this trait. However, we did examine whether the SNP with the strongest association with PR interval, rs7638909, was also associated with QRS duration. We did not find a significant association between *SCN5A* SNPs and QRS duration (p=0.20, Table 2). We also found this SNP to be associated with 2.2 msec (0.19 SD) increased P wave duration ($p=1.5\times10^{-4}$), and 2.6 msec (0.14 SD) increased PR segment for each minor allele copy (p=0.01).

Discussion

Main findings

We have examined the heritability and common genetic determinants of cardiac conduction as measured by electrocardiographic intervals in an isolated founder population. We observed heritability estimates for PR interval similar to previous studies in other populations and significant heritability estimates for P wave duration and PR segment, the subcomponents of PR interval, but not for QRS duration. We performed a genome-wide association study of PR interval and found the SNPs with the lowest p-values to be located in the 3′ end of the gene encoding the alpha-subunit of the cardiac sodium channel *SCN5A*, a strong candidate gene to modulate this electrocardiographic correlate of cardiac conduction. The most strongly associated SNP increases PR interval by 4.8 ms/copy of the minor allele and explains 2.0 % of PR interval variability.

Heritability

The PR interval heritability estimate on Kosrae (34%) is similar to that previously reported in other populations. A family-based study of 1951 individuals of European ancestry from the United States estimated the heritability at 34%.[3] A family-based study in 820 individuals from the Tokelau islands in Polynesia estimated heritability to be 46%.[18] Studies in twins have also shown significant heritability but of varying degree; a study in 355 twin pairs estimated heritability to 34%[5] while a study in 20 Norwegian twin pairs estimated heritability to be 78%.[4] To our knowledge, no previous studies have examined the heritability of PR interval subcomponents P wave duration and PR segment. Studies examining the heritability of QRS interval are scarce but have not demonstrated significant heritability.[5,9] The lack of heritability in these studies could be attributable to limited power to detect such effects due to modest sample sizes or poor precision of QRS measurements, as well as the possibility that additive genetic factors as assessed by heritability do not play much of a role in determination of QRS duration compared to environmental factors.

SCN5A in cardiac conduction

Rare loss-of-function variants in SCN5A have been found to cause delayed conduction with prolonged PR interval and QRS (MIM 600163.0016)[6], atrial fibrillation,[19] sudden cardiac death,[20] sick sinus syndrome (MIM 608567),[21] Brugada syndrome (MIM 601144),[22] dilated cardiomyopathy (MIM 601154),[23] and Progressive Familial Heart Block (MIM 113900).[7]. These variants are likely to result in a reduced action potential upstroke velocity resulting in slowed impulse propagation[24]. Mouse knockout models of *SCN5A* exhibit 50% reduced sodium conductance in patch clamp experiments and impaired atrioventricular and intramyocardial conduction[25,26] whereas transgenic mice with constitutive overexpression of *SCN5A* exhibit shorter P-wave and PR interval.[27] Heterozygous knockout mice were found to resemble the human disease of Progressive Familial Heart Block with a progressive impairment of atrial and ventricular conduction.[28]

Bezzina et al described a common haplotype in the *SCN5A* promoter (immediately 5′ of the gene) of a sample consisting of 102 healthy Japanese subjects and 71 Japanese Brugada syndrome patients without coding *SCN5A* mutations to be associated with PR interval and QRS duration in both groups.[8] We find it unlikely that our association signal is the same as the promoter signal reported by Bezzina et al since the SNPs in our study are all located in the 3′ end of the *SCN5A* gene, a gene which spans 101 kb. No SNP in the 5′ end of *SCN5A* reaches $p<0.33$ and the SNP with the lowest p-value within 50 kb 5′ of the gene has $p=0.05$ (Supplementary Table 1). Second, a recombination hotspot separates our association signal from the SNPs with stronger association in the 5′ end of the gene (Figure 3). Lastly, none of the SNPs within 50kb of the *SCN5A* promoter in HapMap Chinese from Beijing or Japanese

from Tokyo samples or in the Kosraen sample has an r^2 greater than 0.05 to the top SNP identified in the current report (rs7638909). To our knowledge no other studies have described association between common *SCN5A* variants and cardiac conduction. However, previous studies have found association between a common missense variant, H558R, in *SCN5A* and atrial fibrillation in an Asian sample[29] and a variant associated with ventricular arrhythmia, S1102Y, in an African American sample.[30] The H558R variant has low correlation with the top SNP in our report in HapMap (r^2=0.03) and is not included on the Affymetrix 500K GeneChip. The S1102Y variant is very rare in samples of Asian or European ancestry.

As the cardiac sodium channel is expressed widely in both ventricles and atria we also examined the association of *SCN5A* with the ventricular component of cardiac conduction, QRS duration, and did not find any significant association. This might be explained by differences in the properties of atrial and ventricular sodium channels, which have been described previously,[31] lack of power to detect modest genetic effects or a limited genetic contribution to variation in QRS duration. We found the strongest PR interval SNP to be strongly associated with P wave duration, with a weaker association to PR segment, consistent with a general action of the variant identified in both the atrial conduction tissue and the atrioventricular node..

Other GWAS results

For PR interval, the only SNP other than at the *SCN5A* locus to reach a p-value below 10^{-5} is located on chromosome 2, more than 200 kb from any known gene. However, one of the two closest genes is activating transcription factor 2, *ATF-2*, previously implicated in experimental models of cardiac hypertrophy.[32] This finding could merit further examination if replicated in other studies.

For PR segment, the most significant SNP was located near *MSX1*.*MSX1* was recently shown to interact with T-box factors in regulating connexin expression in the heart,[33] which contributes to electrical coupling of cardiomyocytes and has been found to have a role in embryological development of the atrioventricular myocardium making it a strong candidate for follow up.[34] For P wave duration we noted that a SNP in *RYR2*, the calcium channel which couples cardiac muscle excitation to myocardial contraction and is responsible for monogenic cardiomyopathy and ventricular tachycardia, ranks among SNPs with the strongest association.

Strengths and limitations

Genetic screens in isolated populations can be a valuable tool in identifying Mendelian disease genes due to the low genetic and environmental heterogeneity. We have previously shown that genetic heterogeneity in terms of long-range linkage disequilibrium on Kosrae also makes genome-wide association studies with high coverage of common variation feasible, with as much as 80% coverage of all common variation at r^2>80% using only 110,000 SNPs.[12] However, our study is underpowered to fully utilize the greater coverage provided by over 300,000 SNPs in identifying common genetic variants of the low effect sizes that typify many common variants that alter risk of human disease or physiology. The effect size seen in *SCN5A* SNPs is relatively large for a common variant, explaining 2.0 % of trait variability.

The significance thresholds that should be used in genome-wide association studies to control the false-positive rate are a matter of debate. The Wellcome Trust Case Control Consortium employed a threshold of 5×10^{-7}.[35] We have recently recommended a threshold of 5×10^{-8} in European population samples, accounting for roughly 1,000,000 independent common variant tests in the human genome.[36] On Kosrae, it is likely that the increased correlation among SNPs (linkage disequilibrium), as previously shown[12] and exemplified here by the reduced number of haplotypes covering SNPs in *SCN5A* as compared with Asian HapMap samples (Figure 4), makes this threshold overly conservative due to the more limited number of independent tests

across the genome in Kosraens. Lastly, the implication of rare *SCN5A* mutations in Mendelian forms of conduction block and a common variant effect in one Asian study gives greater confidence that the current findings are unlikely to be spurious. Ultimately, proof of true association will need to come from replication in additional samples of various ancestral backgrounds.

The isolated population study design also introduces some potential challenges. The long-range linkage disequilibrium makes fine-mapping and resequencing of identified loci more difficult. In addition, as rare alleles may have risen to high frequencies in founder populations, findings may not be generalizable to other populations. Hence, our finding of *SCN5A* as the top SNP in the PR interval GWAS could be the result of a generally rare variant such as those previously described[6] which has risen to higher frequency on Kosrae as recently shown for phytosterolemia.[37] However, the high minor allele frequency of the variants reported here make this less likely. Further work, including fine-mapping and resequencing, is required to exclude this possibility.

Conclusion

We have identified a common variant at the *SCN5A* locus that influences PR interval in a South Pacific founder population. Replication in other cohorts and extension to clinical samples is needed to define the ultimate clinical implications of these findings. Our results add to results from QT interval duration that systematic genetic scans of ECG traits may identify genes and variants important in electrophysiological function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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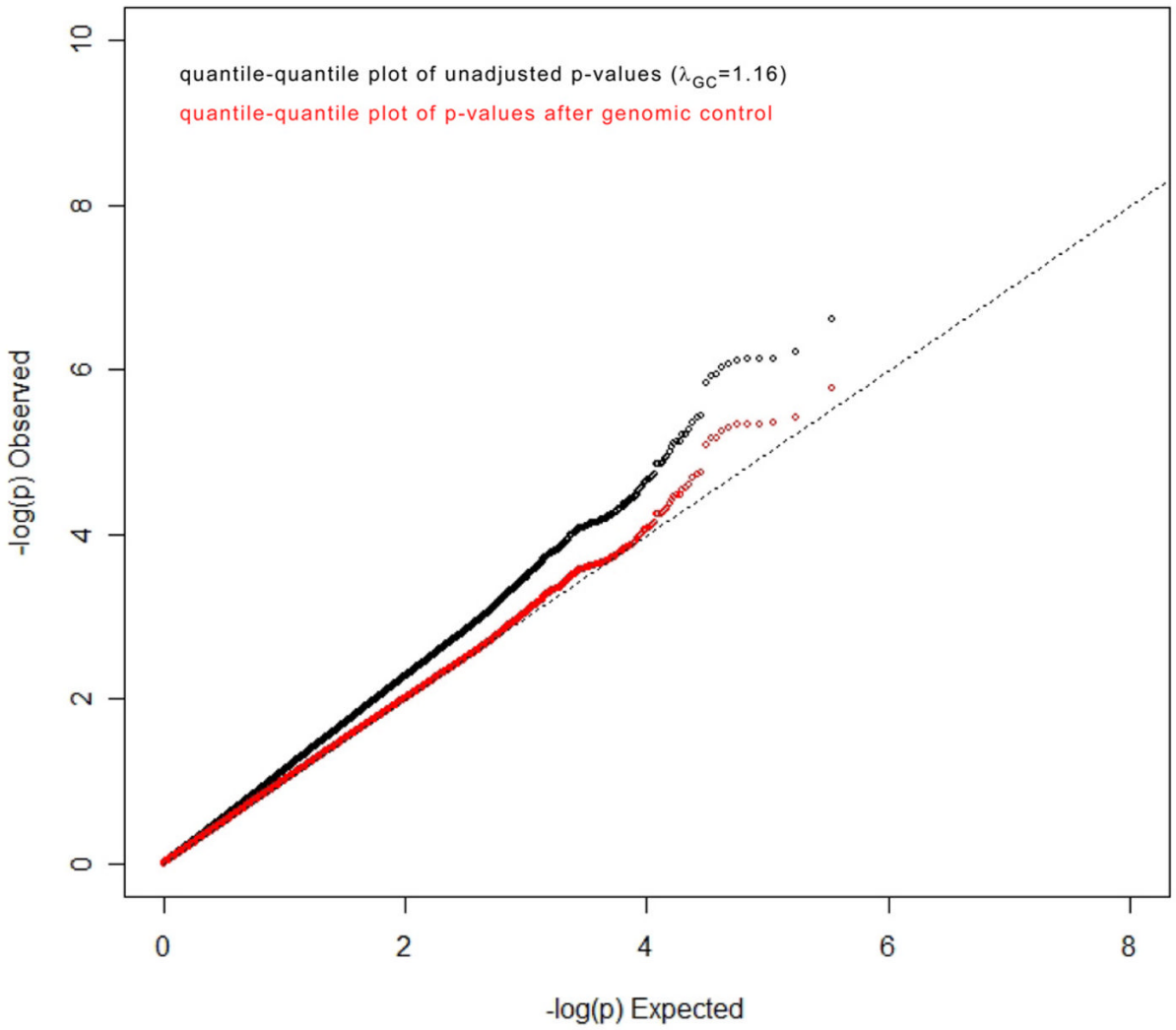

Figure 1.
Quantile-quantile plot of PR interval association results for 338,049 autosomal SNPs under an additive genetic model. Plotted are the expected versus observed $-\log_{10}$(p-value) before (black) and after (red) genomic control from the PR interval GWAS. The measure of overdispersion of the test statistics, $\lambda_{GC,}$ was 1.16 before genomic control.

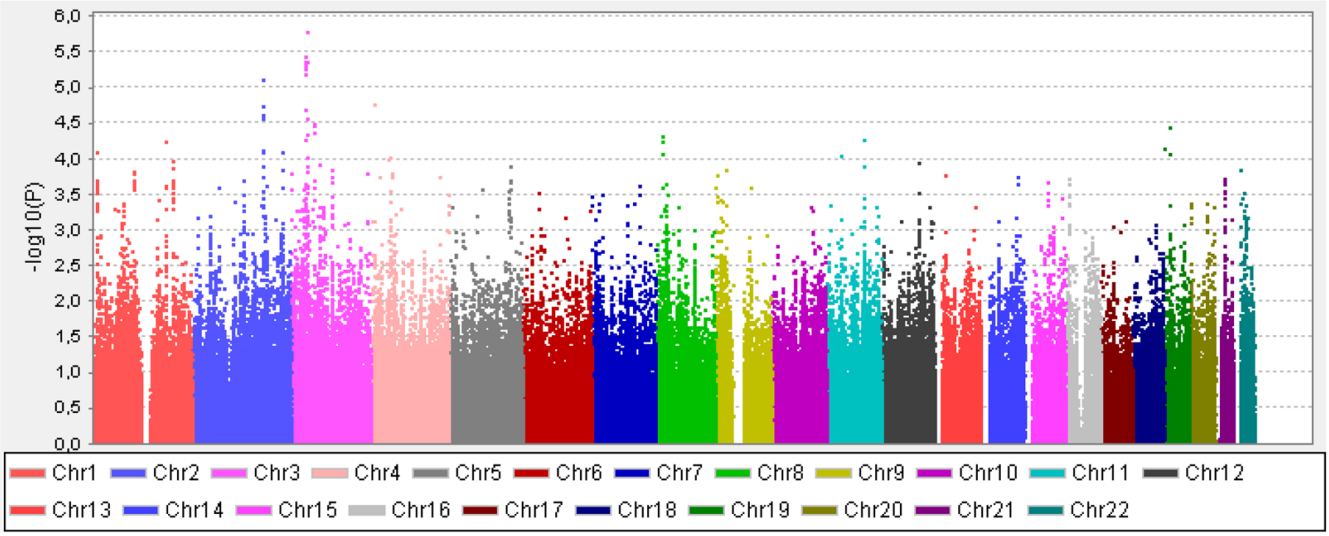

Figure 2.
PR interval association results for 338,049 SNPs across 22 autosomes under an additive genetic model. Each dot represents one SNP. Plotted on the y-axis is $-\log_{10}$(p-value) and on the x-axis physical position by chromosome.

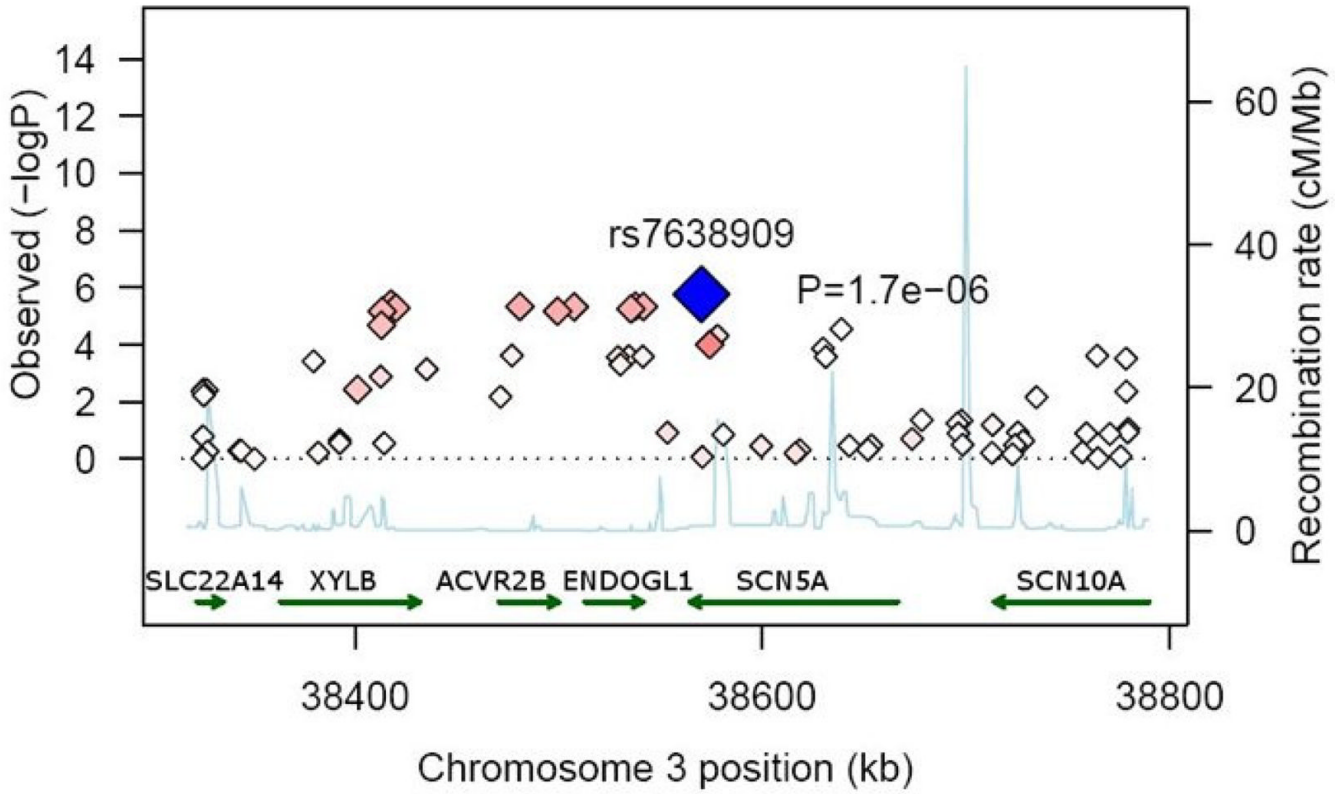

Figure 3.

Regional association plot for PR interval top locus on chromosome 3p. The plot covers the genomic region from 150 kb upstream of *SCN5A* to 300 kb downstream. Positions are from NCBI build 35 and recombination rates as estimated from HapMap phase II. SNPs are represented by diamonds and the large blue diamond is the SNP with the highest p-value. Diamond color represents correlation with the top SNP: faint red indicates weak correlation and brighter red indicates strong correlation. Recombination rate is plotted in the background and known genes in the region are shown in the bottom of the plot.

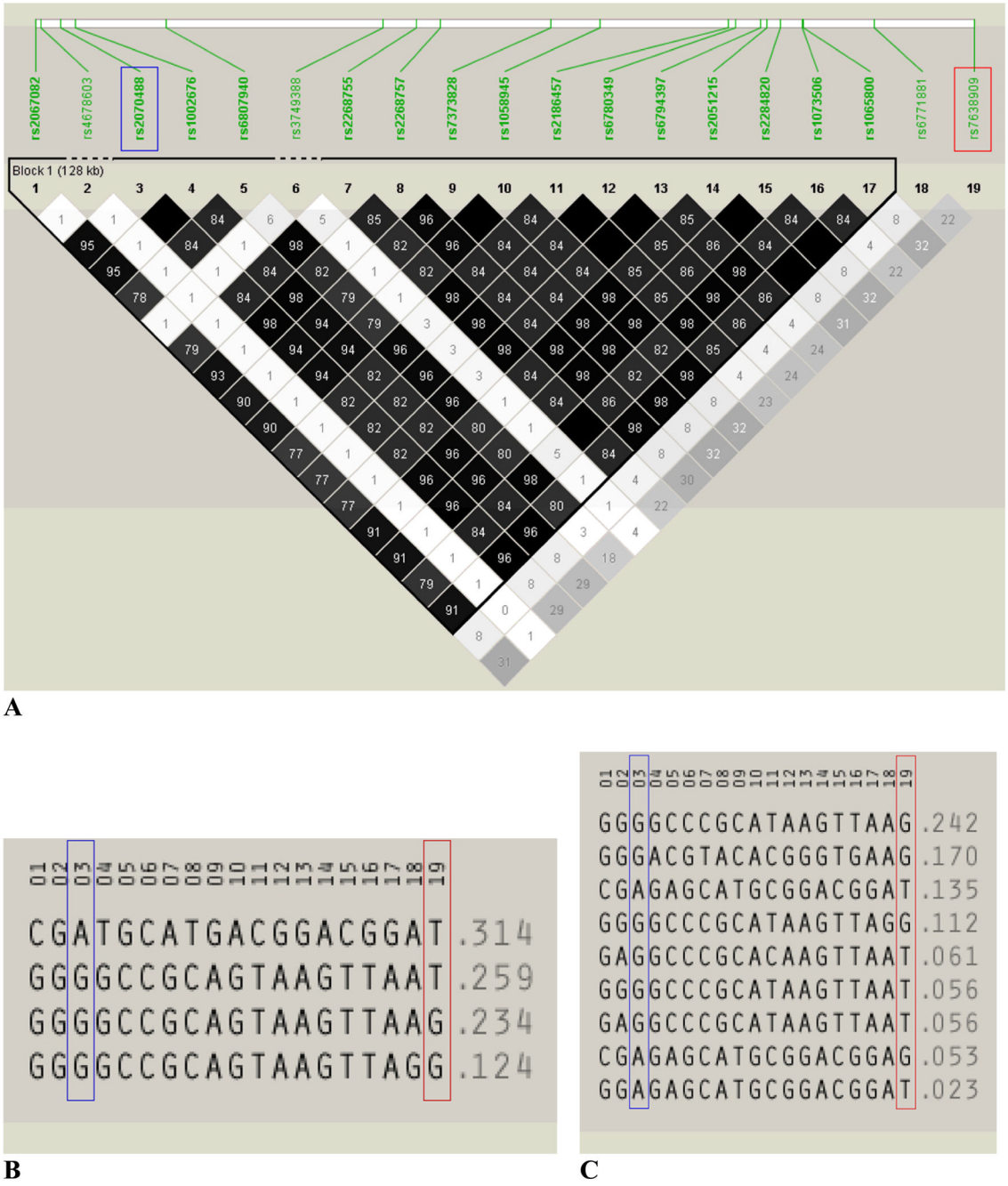

Figure 4.

Linkage Disequilibrium plot showing all SNPs with $p<10^{-5}$ and intervening SNPs in the genomic region of *SCN5A* and downstream. Values in the boxes are pairwise SNP correlations (r^2); box color reflects the degree of correlation. An empty, dark box indicates complete correlation ($r^2 = 100\%$) and a clear box indicates low correlation. The LD block including most of the associated SNPs was defined by the criteria of Gabriel et al as implemented in Haploview[24]. The strongest and second strongest associated SNPs are indicated with red and blue boxes, respectively. Correlations were calculated in the 133 most distantly related Kosraeans (see Methods). Panel B shows haplotypes with all 19 SNPs in the region constructed in Haploview in which each line represents one haplotype followed by its frequency on Kosrae

and the numbers above each column correspond to the number for each SNP as given in the LD plot. Panel C shows haplotypes of the same SNPs from the Asian populations CHB/JPT in HapMap rel21. Haplotypes with frequencies below 0.02 were excluded.

Table 1

Sample characteristics. Mean and standard deviation are reported for each sex and the total sample. Percentage of cases are reported for dichotomous traits.

	Female (n=1049)	Male (n=702)	Total (n=1751)
Age (years)	37.3 (15.3)	37.9 (17.1)	37.6 (16.1)
BMI	31.1 (6.46)	28.9 (5.71)	30.2 (6.26)
SBP (mm Hg)	110.1 (18.2)	115.3 (16.8)	112.1 (17.9)
DBP (mm Hg)	73.4 (11.5)	76.3 (11.1)	74.6 (11.4)
LDL (mmol/l)	2.47 (0.74)	2.60 (0.79)	2.5 (0.76)
HDL (mmol/l)	1.06 (0.26)	0.91 (0.22)	1.0 (0.25)
Chol (mmol/l)	3.88 (0.90)	3.88 (0.95)	3.88 (0.92)
TG (mmol/l)	0.91 (0.42)	1.08 (0.64)	0.98 (0.53)
Type 2 diabetes	16.6 %	16 %	16.3 %
Smoking	0.8 %	28.5 %	11.9 %
PR interval (ms)	164.4 (21.5)	170.1 (24.8)	166.7 (23.0)
QRS interval (ms)	97.4 (9.28)	101.0 (9.19)	98.8 (9.41)
Heart rate (beats/min)	72.1 (9.73)	66.8 (10.1)	69.9 (10.2)
P wave duration (ms)	104.2 (11.7)	107.0 (12.0)	105.3 (11.9)
PR segment (ms)	65.8 (18.7)	67.9 (20.4)	66.6 (19.4)

Abbreviations: BMI = Body Mass Index, SBP = Systolic Blood pressure, DBP = Diastolic Blood Pressure, MAP = Mean Arterial Blood Pressure, LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein, Chol = Total Cholesterol, TG = Triglycerides, FBS = Fasting Blood Sugar

Table 2

Association results from PR interval GWAS with $p<10^{-5}$. Additive genetic models were tested for all 338,049 autosomal SNPs passing quality control. All SNPs happen to be non-coding. Positions are on the forward strand from the reference sequence in NCBI build 35. SNP IDs refer to dbSNP. Alleles are shown as major/minor. MAF refers to minor allele frequency. Effects are milliseconds/copy of minor allele with standard error shown in parentheses. The correlation for each SNP in the chr3 locus with the top SNP as measured by r^2 is based on examination of the 133 most distantly related Kosraeans genotyped (see Methods).

Rank	SNP	Position	Alleles	MAF	PR effect	PR p-value	QRS effect	QRS p-value	r^2
1	rs7638909	Chr3:38569977	T/G	0.40	4.8 (1.0)	1.7×10^{-6}	0.42 (0.34)	0.22	-
2	rs2070488	Chr3:38417494	G/A	0.33	−5.0 (1.1)	3.7×10^{-6}	−0.35 (0.36)	0.33	0.29
3	rs2284820	Chr3:38537583	T/C	0.33	−4.9 (1.1)	4.4×10^{-6}	−0.37 (0.37)	0.33	0.32
4	rs1065800	Chr3:38541484	A/G	0.33	−4.9 (1.1)	4.6×10^{-6}	−0.31 (0.36)	0.38	0.32
5	rs2268757	Chr3:38480857	C/T	0.33	−5.0 (1.1)	4.6×10^{-6}	−0.34 (0.37)	0.36	0.30
6	rs1058945	Chr3:38507515	G/A	0.33	−4.9 (1.1)	4.7×10^{-6}	−0.32 (0.36)	0.36	0.32
7	rs1002676	Chr3:38419936	G/T	0.33	−4.9 (1.1)	5.1×10^{-6}	−0.35 (0.38)	0.35	0.29
8	rs2051215	Chr3:38535349	G/A	0.33	−4.9 (1.1)	5.5×10^{-6}	−0.33 (0.36)	0.36	0.32
9	rs7373828	Chr3:38499315	A/G	0.33	−4.9 (1.1)	6.7×10^{-6}	−0.32 (0.35)	0.35	0.33
10	rs2067082	Chr3:38413186	G/C	0.34	−4.8 (1.1)	6.7×10^{-6}	−0.22 (0.38)	0.56	0.31
11	rs2461751	Chr2:176114826	A/G	0.44	4.54 (1.0)	8.0×10^{-6}	-	-	-