SUPPLEMENTAL MATERIAL

Expanded methods

QTc-interval measurement

ECGs were digitalized and analyzed using ImageJ (http://rsb.info.nih.gov/ij/) blinded to genotype. Only sinus rhythm complexes were analyzed. The QT-interval was measured manually on-screen using the tangent method.19 Lead II was used whenever possible. The average QT-interval from up to 3 consecutive beats with similar preceding RR-intervals was calculated and the QTc-interval was expressed using Bazett's formula ($QTc = QT/\sqrt{RR}$).

Genotyping

Candidate gene SNPs

SNP genotyping was performed using a custom assay (Illumina GoldenGate) on an Illumina-BeadStation500GX. The Illumina BeadStudio software clustering algorithm was used for initial data analysis. Thereafter, intensity plots of all variants were examined individually and manual genotype calling was performed if necessary. Monomorphic SNPs and SNPs with a MAF < 1%, as well as SNPs with call rates <95% were removed from further analyses. A total of 1,201 SNPs had sufficient quality for analysis. All patients included in the association analysis had call rates ≥95%.

SNPs from QTc-interval GWAS

Genotyping was performed on the MassARRAY system using MALDI-TOF mass spectrometry with the iPLEX Gold chemistry (Sequenom Inc, San Diego, CA, USA). Primers were designed using Assay Designer 4.0.0.2 with iPLEX Gold default parameters. Automated genotype calling

was done with Typer Analyzer 4.0.22.67. An experienced evaluator checked genotype clustering visually. The 3 SNPs (rs16847548, rs1935778, rs956642) from the candidate gene study genotyped in patient Set 2 were also typed in this way.

Statistical significance thresholds

For the candidate gene study, the Bonferroni-corrected statistical significance threshold was set at $p=4.2\times10^{-5}$ (0.05/1,201) and p=0.016 (0.05/3), for the discovery and the replication phase, respectively. In testing the SNPs from GWAS, the Bonferroni-corrected significance threshold was set at $p=2.27\times10^{-3}$ (0.05/22), both for the quantitative effect analysis on QTc-interval as well as in the case-control analysis. In the analysis for effects on cardiac events, a p-value threshold of 0.004 (0.05/12) was used.

Power statement

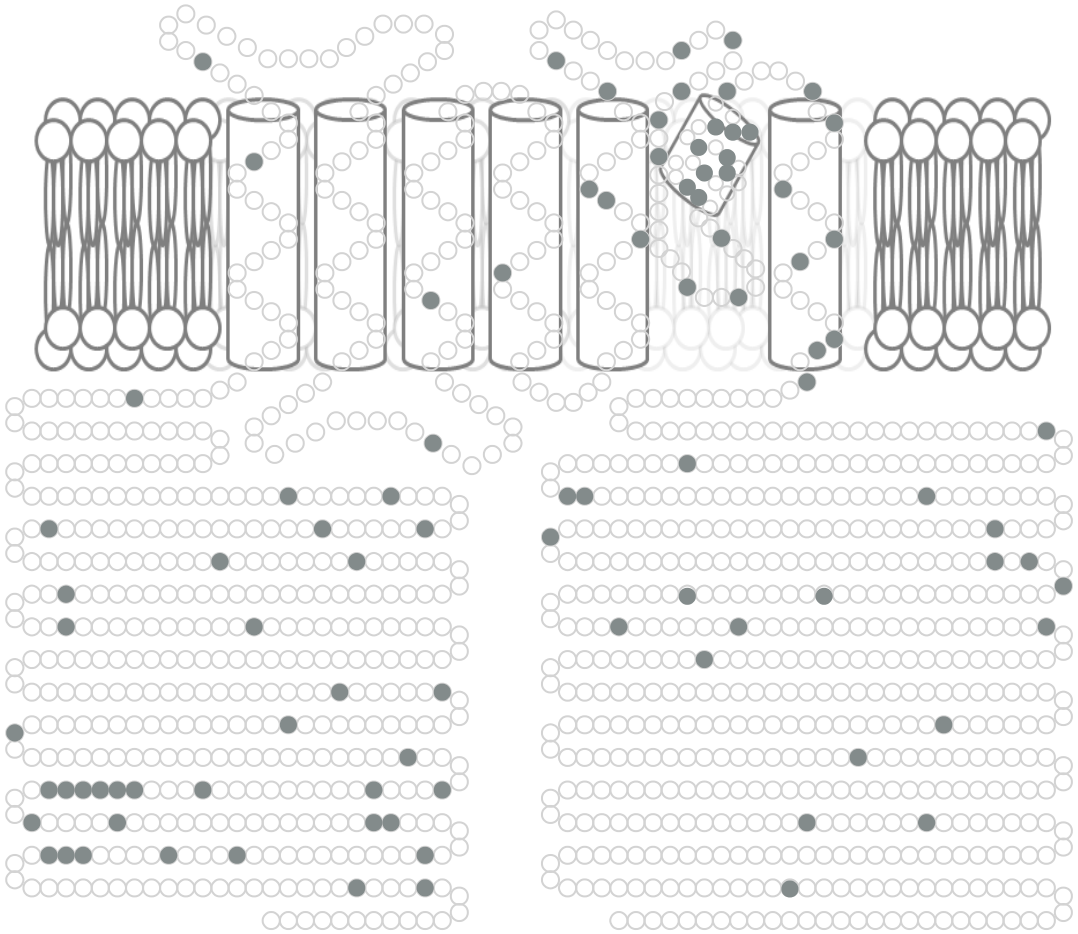
With the present sample sizes, QTc effect sizes of 28, 19 and 17 ms could be detected in the candidate gene study with 80% power for SNPs with minor allele frequencies of 0.1, 0.25 and 0.5, respectively. For the SNPs from the GWAS, effect sizes of 17, 12 and 10 ms could be detected with 80% power for SNPs with minor allele frequencies of 0.1, 0.25 and 0.5, respectively. For both, we assumed a mean (±SD) QTc of 465 ± 45 ms. For the cardiac events, we could detect hazard ratios of 1.9, 1.6 and 1.5 for SNPs with minor allele frequencies of 0.1, 0.25 and 0.5, respectively, assuming an event rate of 32%. For the case-control study, we could detect odds ratios of 2.1, 1.8 and 1.70 for SNPs with minor allele frequencies of 0.1, 0.25 and 0.5, respectively. For all calculations, we assumed a (log) additive genetic model and two sided significance thresholds as described in the previous section.

Supplementary Table 1: Overview of candidate genes and SNPs tested

Gene	Chromosome	Tag SNPs passing QC
AKAP9	7q21-q22	14
ANK2	4q25-q27	103
CACNA1C	12p13.3	150
CASQ2	1p13.3-p11	59
CAV3	3p25	55
FKBP1B	2p23.3	12
GPD1L	3p22.3	40
KCNE1	21q22.1-q22.2	57
KCNE2	21q22.1	24
KCNH2	7q35-q36	58
KCNJ2	17q23.1-q24.2	37
KCNQ1	11p15.5	112
NOS1AP	1q23.3	110
RYR2	1q42.1-q43	201
SCN1B	19q13.1	22
SCN4A	17q23.1-q25.3	22
SCN4B	11q23	45
SCN5A	3p21	70
Total		1191

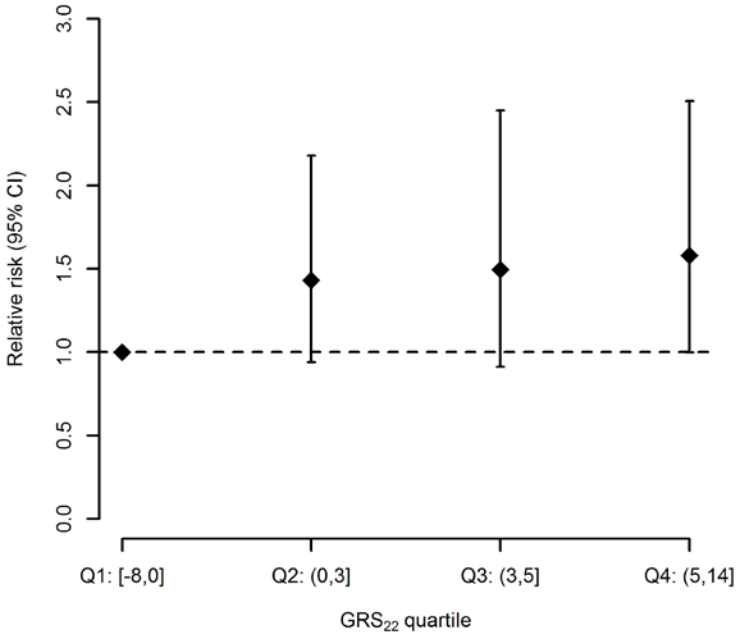
Tag SNP, haplotype-tagging SNP

Supplementary Table 2: A list of the effect of all SNPs from the 18 candidate genes on the QTc-interval in Patient Set 1, available in a separate Excel (xlsx) file that accompanies this supplemental file.

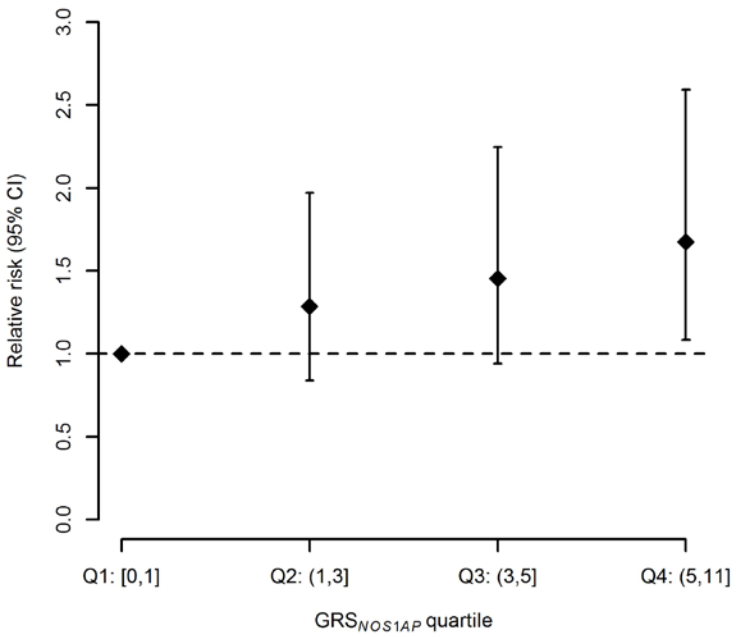


Supplementary Figure 1: Location of the 128 different missense mutations in the Kv11.1 (HERG) potassium channel encoded by the *KCNH2* gene. The transmembrane S1-S4 region was defined as amino acid residues 404 to 547. The transmembrane loop region was defined as residues 548 to 659. Residues 1-403 were defined as N-terminus, while residues 660 to 1159 were defined as the C-terminus. The cylinders represent putative α-helical segments, and the bars represent putative β-sheets. These annotations are based on the Uniprot database (http://www.uniprot.org/uniprot/Q12809, accessed January 2012).

A

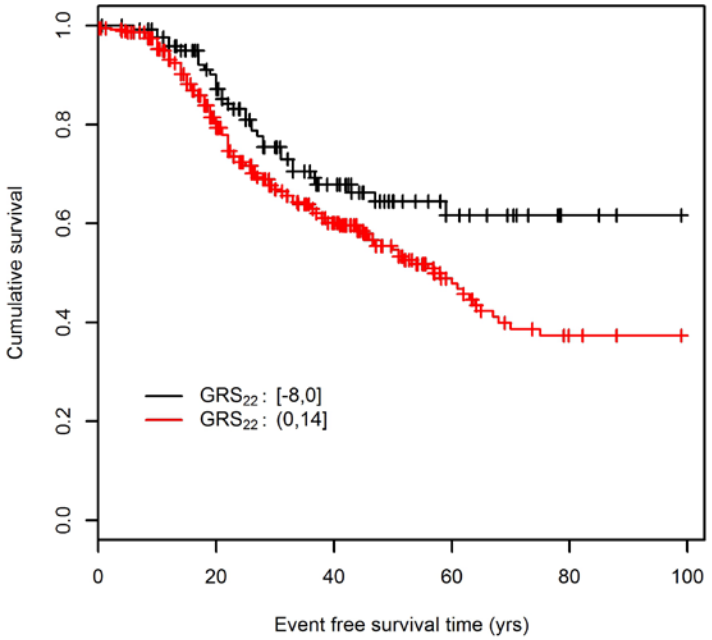


B

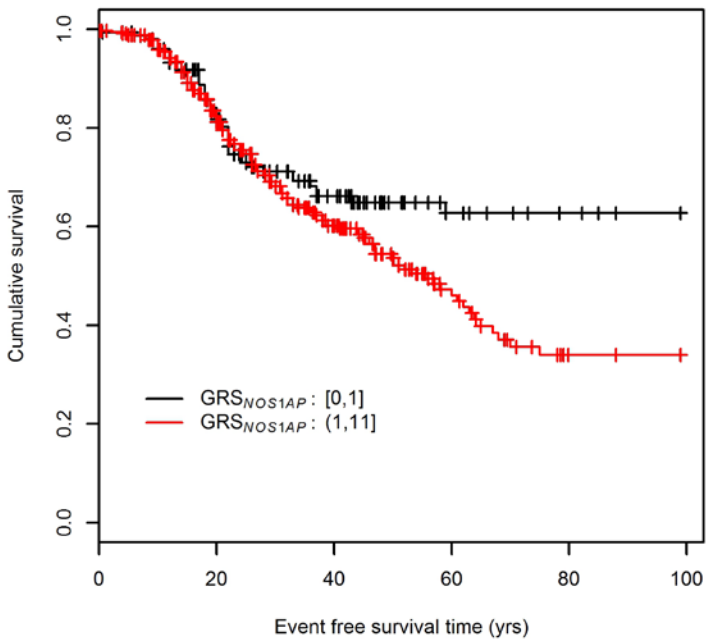


Supplementary Figure 2A, B: Relative risks (95% CI) of GRS quartiles 2, 3 and 4 vs. quartile 1 for GRS_{22} and GRS_{NOS1AP}.

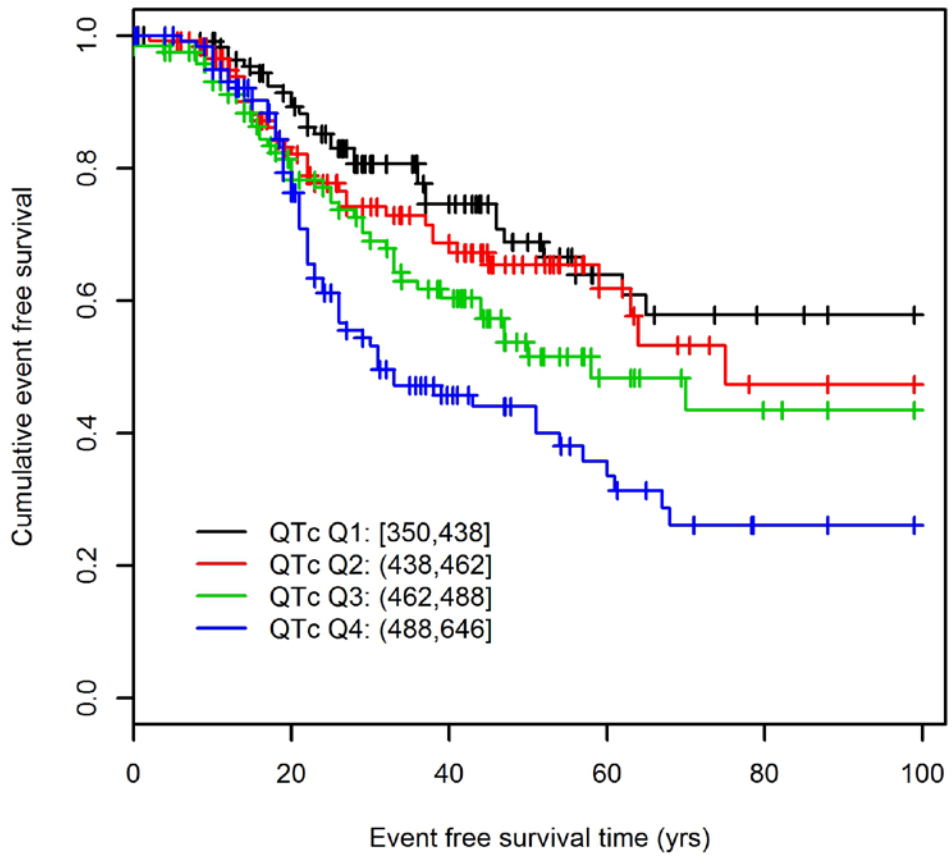
A



B



Supplementary Figure 3A, B: Cumulative event-free survival in LQTS patients with GRS scores in quartile 1 vs quartiles 2, 3 and 4 combined for GRS_{22} and GRS_{NOS1AP}.



Supplementary Figure 4: Association between the QTc-interval and event-free survival. The QTc-interval was a strong predictor of cardiac events with patients with a QTc-interval in the highest quartile (Q4) having a RR of 2.11 (95% C.I. 1.35-3.30) as compared to patients in the lowest QTc-interval quartile ($p=7.9 \times 10^{-7}$).