SUPPLEMENTAL MATERIAL

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A. Methods

Participant protocol

Carriers of c.671C>T (p.T224M) KCNQ1 variant

The study was approved by the Institutional Review Board of the University of Maryland School of Medicine. Our research ES consent form included permission to recontact participants but did not include permission to disclose specific genetic results or to perform automatic clinical confirmation of a variant. Participants in this study were Old Order Amish (Amish) who live in Pennsylvania, most in Lancaster County, PA. The population of Amish in Lancaster County is estimated to be approximately 30,000. For the 5,521 participants included in this study, the mean age was 41.7 years, 56.4% female, 5.8% (312/5521) had an abnormally high QTc (>450 ms for men, >460 ms for women) and 17 (0.3%) had a QTc above 500 ms (Supplemental Table 1). Most Amish do not have telephones in their homes, so communication with them was done by mail. An initial letter was sent to the 124 individuals identified by research ES to carry the c.671C>T (p.T224M) *KCNQ1* variant, indicating that a genetic change had been identified in them that could put them at risk for a heart problem, without providing further details. This letter was reviewed by our Amish liaisons (Amish women who help with study recruitment and community relations) for understandability and content. If

interested, individuals were asked to schedule an in-home visit. If not interested, they were asked to return an enclosed letter, checking off or writing in the reason that they were not interested in obtaining more information.

The protocol consisted of two in-home visits. On visit 1, a medical geneticist and an Amish liaison met with participants in their homes. After obtaining written informed consent, a full medical history was obtained by the geneticist, including current medications, past medical history, history of syncope (describing each event, including whether it occurred during rest or activity or had vasovagal qualities such as during phlebotymy), and cardiac symptoms (palpitations and lightheadedness). A threegeneration pedigree was recorded including questions regarding details on crib death and SCD in family members. Blood was drawn for CLIA confirmation of the *KCNQ1* variant and an EKG was obtained both supine and within 10 seconds of standing. The latter was performed because the QTc can increase significantly immediately after standing in those with LQTS and can increase the sensitivity of diagnosing LQTS
clinically[1,2]. Performing stress tests was not feasible. The Schwartz score was calculated[3,4] in all 88 carriers and 54 non-carriers to determine how many individuals with the variant met the clinical criteria for LQTS without consideration of the p.T224M variant.

Syncope was defined as loss of consciousness that was unexpected (eg. not associated with an accident or other physical injury). Sudden deaths in family members were labeled SCD when they were unexpected or not the result of known disease (eg.

malignancy, coronary artery disease and genetic syndromes enriched in the Amish such as Ellis van Creveld syndrome, microcephaly, cartilage hair hypoplasia, or inborn errors of metabolism). Medical records and death certificates of family members with sudden death including crib death were not available, nor was DNA for genotyping for the p.T224M variant. In all cases of crib death and SCD in children, families indicated that autopsies were performed and were normal but these reports were not available. In cases of SCD in adults, autopsies were generally not performed.

After the QTc was measured on EKGs by an electrophysiology cardiologist and CLIA confirmation of the variant was performed, a letter was sent to participants requesting scheduling of visit 2. At in-home visit 2, the medical geneticist discussed the *KCNQ1* results and provided genetic counseling, including recommendations that first degree family members be tested for the *KCNQ1* variant. The nearby Clinic for Special Children, in Strasburg, PA offers clinical genotyping for many disease variants enriched in Anabaptist populations[5]. In addition, after discussion with the cardiologist, recommendations were made to participants for beta-blocker treatment using the Heart Rhythm Society guidelines[6]. The guidelines recommend beta-blocker treatment in individuals with LQTS diagnosed either clinically or by having a pathogenic mutation associated with a QTc $\geq$470 ms regardless of symptoms and in those who have QTc <470 ms who have also had a syncopal event or cardiac symptoms that are believed to be due to LQTS. For *KCNQ1* related LQTS, nadolol has been shown to be the most effective beta-blocker[7]. Nadolol was recommended for those who met the criteria for treatment except for women of child bearing age, in whom propranolol was recommended because of its greater safety in pregnancy and nursing[8]. Nadolol is

transferred into breast milk to a greater degree than other beta-blockers and is considered contraindicated during nursing. After visit 2, a final letter was mailed to participants including a full review of what was discussed during the visit, copies of the participant EKG, clinical *KCNQ1* c.671C>T (p.T224M) variant testing report, specific recommendations on beta-blocker treatment, and recommendations for cascade testing in first degree relatives. A "Dear Doctor" letter was also included for the participant to give to his/her primary care practitioner (PCP) with beta-blocker treatment recommendations. For those who requested, the letter was also sent directly to his/her PCP. With all letters, a list of medications known to prolong QTc was included. There were 5 individuals who failed to schedule home visit 2. For them, a 2nd letter was sent 3 months later, again requesting to schedule a visit. If no response was received within 6 months of home visit 1, the same "final letter" and "doctor letter" were mailed to these participants.

Non-carrier participants

Non-carriers used as comparators to c.671C>T (p.T224M) carriers were sequential participants of the Amish Wellness Study, a community based study eligible to all Amish adults. Questions pertinent to LQTS included history of syncope (with description and circumstances, whether with activity or at rest), cardiac symptoms (palpitations and lightheadedness), and family history of SCD in first degree relatives at different ages. Following the previously described criteria as for carriers, known causes of sudden death including accidents, malignancy and genetic syndromes known to increase the risk of sudden death were excluded. In addition, an immediate standing EKG, as done in carriers, was added to the supine EKG that was standard in the Wellness Study.

To increase the power of comparing family history of SCD between *KCNQ1* carriers and the 54 non-carriers who had standing EKGs, additional non-carriers from our Amish studies database were included who had information available about personal history of syncope and family history of sudden death. These questions were added to the Wellness Study protocol during the *KCNQ1* study and at the time of this publication answers were available for an additional 83 non-carriers for a total of 137 non-carriers. These additional 83 non-carriers were not included in the comparison of QTc interval to carriers because their QTc were not measured manually by an electrophysiologist as was done for the initial 54 non-carriers.

Measurement of QTc

QTc was measured manually by an electrophysiologist using the tangent method. Twelve-lead electrocardiography (EKG) was performed at 25 mm/s sweep speed and 10 mm/mV amplitude in a supine position and within 10 seconds after standing. The QT interval was defined as the time from the onset of the QRS complex to the end of the insection of the tangent of the steepest slope of the last limb of the T wave and the TP isoelectric baseline[6,7,9,10]. Corrected QT (QTc) intervals for heart rate were calculated using Bazett's correction formula ($QTc = QT/\sqrt{\overline{RR)}}$ using the preceding RR interval of each QT measurement[4,10-13]. All QT intervals were measured in 2 leads (lead II and lead V5). If either lead was not available due to artifact, the most consistent limb and precordial lead with the longest QT interval were used. QT intervals were measured for 3-5 consecutive beats in sinus rhythm during periods of no ectopic beats or other arrhythmias. Five consecutive beats were measured for at least 1 lead per 12-lead

EKG. The QTc for each lead was reported as the mean of the 3 - 5 measured intervals. Both mean and maximal QTc were determined and are reported. The maximal QTc was based on the greater of the two mean QTc values for either of both measured leads. The Schwartz score was calculated using supine maximal QTc, using the modified Schwartz score formula[3,4].

Exome sequencing (ES)

Whole blood was collected for ES. In some cases, stored DNA was used, collected up to 20 years earlier. Exome capture and sequencing were performed in collaboration with the Regeneron Genetics Center (RGC). Exome capture was performed using xGen Lockdown Design Tool available from IDT with some modifications. The captured libraries were sequenced on the Illumina HiSeq 2500 platform with v4 chemistry using paired-end 75 bp reads. Paired-end sequencing of the captured bases was performed so that >85% of the bases were covered at 20x or greater. Read alignment and variant calling were performed using BWA-MEM and GATK as implemented in the RGC DNAseq analysis pipeline. SNPs with call rate <90%, and monomorphic SNPs were excluded. SNPs on the Y chromosome and the mitochondrial genome were also excluded. Samples failing QC metrics for contamination, high level of Mendelian errors, identical or MZ twins (one of each pair), MZ quadruplets (three of four) and sex mismatch were excluded.

Clinical confirmation of c.671C>T (p.T224M) *KCNQ1* variant

Clinical confirmation of the c.671C>T (p.T224M) variant was performed in the Translational Genomics Laboratory (CLIA # 21D2027356, CAP# 8017554) at the

University of Maryland School of Medicine. Exon 5 of the RefSeqGene (exon 4 of NM_000218.2) of *KCNQ1* was analyzed by bidirectional Sanger DNA sequencing from whole blood using Sequencher sequencing analysis software (version 5). Sequences were compared to NCBI reference sequences for *KCNQ1 (*NG_008935.1, NM_000218.2 and NP_000209.2).

Functional studies

Site-directed mutagenesis for the c.671C>T (p.T224M) variant was performed on a plasmid containing a 710 bp region (encoding amino acids #1 - 237) of *KCNQ1* with the QuikChange Lightning Multi-site kit (Agilent) using the primer 5'GCAGGTGTTTGCCATGTCGGCCATCAGGG3'. The mutated region of *KCNQ1* was subcloned using restriction enzymes ClaI and Bsu36I into an expression vector (pIRES2-EGFP) containing full-length *KCNQ1*:IRES:GFP, and the wild-type and mutant sequences were confirmed. Plasmids were transfected into CHO cells using Fugene 6 (Promega) following manufacturer's instructions. Wildtype or p.T224M pIRES2-GFP *KCNQ1* plasmids were co-transfected in equimolar ratios with a pIRES2-dsRed expression plasmid expressing wildtype *KCNE1* (the I_{Ks} accessory subunit). Two days post transfection, cells expressing both *KCNQ1* and *KCNE1* were identified by selecting cells that fluoresced green and red, and were patch clamped[14].

Whole cell voltage clamp experiments were performed at room temperature (22 - 23˚C) with 3~5 mΩ patch microelectrodes, using a MultiClamp 700B amplifier (Molecular Devices Inc., Sunnydale, California). The extracellular solution contained (in mmol/L) NaCl 145, KCl 4.0, $MgCl_2$ 1.0, CaCl2 1.8, glucose 10, and HEPES 10; the pH was 7.4,

adjusted with NaOH. The pipette (intracellular) solution contained (in mmol/L) KCl 110, $MgCl_2$ 1.0, ATP-K_2 5.0, BAPTA-K4 5.0, and HEPES 10; the pH was 7.2, adjusted with KOH. Data acquisition was performed using pClamp 10.7 (Molecular Devices Inc.), sampling at 1 kHz and low-pass-filtered at 5 kHz. Activating current was elicited with 5second depolarizing pulses from a holding potential of −80 mV at 20-mV increments, and tail current was recorded on return to −40 mV. The voltage-clamp protocol is shown in Figure 3. Pulses were delivered every 30 seconds. I-V relationships were analyzed by fitting the Boltzmann equation to the data: $I = I_{max}/\{1+\exp[(V_t-V_{0.5})/k]\}$, where I_{max} is the maximal current, V_t is the test potential, $V_{0.5}$ is the membrane potential at which 50% of the channels are activated, and k is the slope factor. Current densities (pA/pF) were obtained after normalization to cell surface area calculated by the Membrane Test in pClamp 10.7.

Statistical analyses

Both phenotypes and genotypes were carefully curated prior to the analysis. Five subjects with QTc interval values below 200 ms were excluded as not being physiological, likely the result of data input error. Genotype quality control was performed with PLINK2[15]. Variants with missingness greater than 10% and subject samples with missingness greater than 10% were removed. Mendel error rates were computed and variants or subjects with Mendel error rates greater than 5% were removed using the “variant first” option. Both duos and trios were considered in the Mendel error rate filtering[16-18]. For exome-wide association analysis, a linear mixed model, single-variant analysis was performed with our in-house MMAP software[19].

Covariates in the analysis included age, sex, and a polygenic component for family structure. The polygenic component was modeled using a genomic relationship matrix (GRM) constructed by the MMAP program using the Amish Illumina Global Screening Array genotypes. The genotypes were pruned using PLINK2[15] and the following parameters: minor allele frequency <0.01, LD $r^2 < 0.1$, 1 Mb window with 100 kb overlap. The analysis results were explored with the Omics Analysis, Search and Information System (OASIS), available from: https://edn.som.umaryland.edu/OASIS[20], a web-based application for mining of association results using a broad spectrum of functional annotation and online resources. The OASIS tool also has links to ANNOVAR, GTEx and NCBI[16-18].

We computed polygenic risk scores (PRS) for all study participants to estimate their genetic risk for having higher QT intervals. PRS were based on the number of risk alleles at 39 loci identified as being associated with QT interval in two prior GWAS[21,22]. The contribution of each risk allele was weighted by its effect size in the reference GWAS and calculated using the PRSice software[23]. The associations of PRS scores on QTc were estimated using linear mixed models[19] with age and sex as covariates. The 39 SNPs included in the PRS are listed in Supplemental Table 3, along with the risk allele frequencies and effect sizes on QT interval from both the reference GWAS and in the Amish.

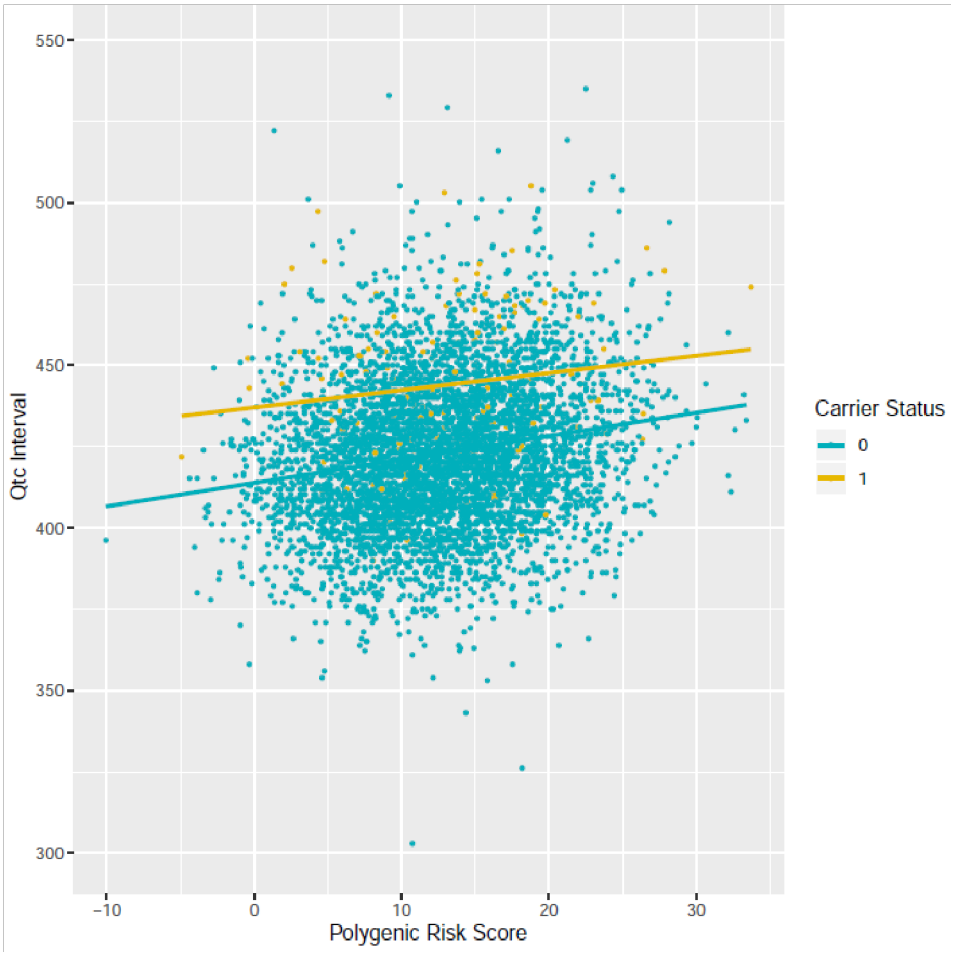
To evaluate possible modifying effects of variants in other genes reported to affect QTc (in other *KCNQ1* pathogenic variants) on the Amish-enriched p.T224M variant, we assessed the effects of the p.T224M variant on QTc in subjects with and without each of

these common modifying variants and tested for p.T224M*modifying variant interaction on QTc using a linear mixed model (Supplemental Table 4).

For whole-cell voltage clamp study, 2 way Anova was used to compare the differences between activating and deactivating currents at each tests membrane potential in the two groups of cells (wild-type and mutant). The results were expressed as mean ±SEM, with significance levels ($p < 0.05$ or $p < 0.01$) reported in the text or figure.

B. Supplemental Figures

Supplemental Figure 1. Scatter plot showing regression of PRS on QTc in *KCNQ1* carriers and noncarriers.



C. Supplemental Tables

Supplemental Table 1. Summary QTc interval statistics for all 5,521 Amish subjects (both p.T224M carriers [CC] and non-carriers [CT] (Panel A). Values by genotype for p.T224M and sex (Panel B).

A.

Variable	Total	Male	Female
N (%)	5521	2407 (43.6)	3114 (56.4)
Age ± SD, years	41.7 ± 15.2	42.7 ± 14.8	40.9 ± 15.5
Mean QTc ± SD, ms	423.4 ± 22.9	411.8 ± 20.4	432.4 ± 20.7
Abnormal QTc*, n (%)	346 (6.3)	95 (3.95)	251 (8.06)
QTc >500 ms, n (%)	19 (0.34)	3 (0.12)	16 (0.51)

*Abnormal QTc for males >450 ms and females >460 ms.

B.

	Carrier Total	Carrier Male	Carrier Female	Non-carrier Total	Non-carrier Male	Non-carrier Female	p-value C vs NC Total	p-value C vs NC Male	p-value C vs NC Female
N (% female)	124 (55.6)	55	69	5397 (56.4)	2352	3045	0.86		
Age, years (±SD)	39.9 (15.7)	40.7 (14.6)	39.3 (16.5)	41.7 (15.2)	42.8 (14.8)	40.9 (15.5)	0.19	0.30	0.40
Mean QTc, ms (±SD)	444.0 (22.6)	429.8 (17.1)	455.2 (19.9)	422.9 (22.7)	411.4 (20.4)	431.8 (20.4)	<0.0001	<0.0001	<0.0001
Abnormal QTc*, n (%)	34 (27.4)	6 (10.9)	28 (40.6)	312 (5.8)	89 (3.8)	223 (7.3)	<0.0001	0.011	<0.0001
QTc >500, n (%)	2 (1.6)	0	2 (2.9)	17 (0.3)	3 (0.1)	14 (0.5)	0.029	0.99	0.015

*Abnormal QTc for males >450 ms and females >460 ms. C = carrier; NC = non-carrier of pT224M *KCNQ1.*
Means and standard deviations obtained by t-tests and chi-square tests.

Supplemental Table 2. Single variant analysis of 6 variants at *KCNQ1* locus with QTc (Panel A) and conditional analysis showing association of each variant with QTc after accounting for rs199472706 (p.T224M)(Panel B). Each conditional analysis model tests the association of the linkage disequilibrium (LD) SNP with rs199472706 included in the model (and with adjustment for age and sex). A.

			Single Variant Analysis			Linkage Disequilibrium	
rsNum	**Chr**	**Position**	**Beta**	**SE**	**P**	**r^2**	**D-prime**
rs199472706	11	2,592,621	20.23	1.99	5.53E-24	1.000	1.000
rs41442048	11	1,080,917	17.82	2.09	1.78E-17	0.619	0.836
rs543805679	11	1,643,226	16.97	2.15	3.17E-15	0.571	0.814
rs41359951	11	1,079,746	12.74	1.85	6.59E-12	0.458	0.738
rs756771432	11	862,837	13.95	2.45	1.31E-08	0.414	0.753
rs201057210	11	1,080,880	8.15	1.49	4.94E-08	0.281	0.730

B.

			Conditional Analysis		
rsNum	**Chr**	**Position**	**Beta**	**SE**	**P**
rs199472706	11	2,592,621	-	-	-
rs41442048	11	1,080,917	3.10	3.38	3.60E-01
rs543805679	11	1,643,226	2.17	3.16	4.92E-01
rs41359951	11	1,079,746	-0.44	2.55	8.62E-01
rs756771432	11	862,837	1.55	2.85	5.86E-01
rs201057210	11	1,080,880	0.47	1.73	7.86E-01

Supplemental Table 3. 39 SNPs included in polygenic risk score based on their association with QTc in two prior GWAS meta-analyses.

SNP	Chr	Pos (hg19)	Coded allele	Noncoded allele	Coded allele frequency	Beta	SE	P-value	Closest gene	Amish Beta	Amish SE	Amish pvalue	Amish MAF
rs11121483	1	6263792	G	A	0.39	1.51	0.22	5.49E-12	RNF207	1.89	0.46	4.88E-05	0.46
rs2298632	1	23710475	T	C	0.50	0.70	0.09	1.40E-14	TCEA3	1.54	0.51	2.58E-03	0.70
rs6588213	1	67107894	T	C	0.13	1.60	0.28	1.53E-08	SGIP1	1.17	0.63	6.11E-02	0.16
rs142804708	1	162012135	T	C	0.05	-5.35	0.87	7.59E-10	OLFML2B	2.00	2.58	4.38E-01	0.01
rs12143842	1	162033890	T	C	0.24	3.50	0.11	1.30E-213	NOS1AP	3.45	0.52	2.91E-11	0.29
rs59852339	1	162112966	C	T	0.11	3.21	0.30	1.16E-26	NOS1AP	1.64	0.79	3.90E-02	0.09
rs12567315	1	162166646	A	G	0.20	3.33	0.23	2.91E-48	NOS1AP	2.83	0.51	3.71E-08	0.29
rs115263373	1	162169913	G	T	0.06	-2.36	0.40	4.41E-09	NOS1AP	-2.76	0.83	9.14E-04	0.09
rs77915002	1	168686870	G	A	0.06	2.17	0.39	3.30E-08	DPT	3.07	0.82	1.82E-04	0.09
rs1200118	1	169064630	G	A	0.48	-1.37	0.24	8.10E-09	ATP1B1	-2.22	0.93	1.70E-02	0.07
rs10919070	1	169099037	C	A	0.13	-1.68	0.14	1.10E-31	ATP1B1	-2.51	1.01	1.29E-02	0.06
rs12997023	2	40752982	C	T	0.05	-1.69	0.22	4.70E-14	SLC8A1	-0.32	1.10	7.74E-01	0.05
rs7561149	2	179689856	C	T	0.42	-0.52	0.09	7.10E-09	TTN-CCDC141	-0.65	0.46	1.59E-01	0.59
rs295140	2	201160699	T	C	0.42	0.61	0.08	4.40E-13	SPATS2L	-0.31	0.48	5.17E-01	0.38
rs6793245	3	38599037	A	G	0.32	-1.12	0.10	4.40E-27	SCN5A-SCN10A	-0.33	0.50	5.18E-01	0.32
rs17784882	3	47544003	A	C	0.40	-0.54	0.10	3.30E-08	C3ORF75	-0.14	0.47	7.58E-01	0.57
rs2363719	4	72138216	A	G	0.11	0.97	0.16	7.80E-10	SLC4A4	1.06	0.68	1.22E-01	0.14
rs11097788	4	103407428	G	A	0.56	1.05	0.19	1.81E-08	NFKB1	0.55	0.47	2.45E-01	0.56
rs10040989	5	137573725	A	G	0.13	-0.85	0.13	5.40E-11	GFRA3	-1.16	0.79	1.41E+00	0.10
rs11153730	6	118667522	C	T	0.50	2.25	0.18	1.80E-35	SLC35F1	1.09	0.47	1.96E-02	0.42
rs6911599	6	119009913	G	A	0.50	-2.13	0.18	1.60E-31	CEP85L	-1.06	0.47	2.46E-02	0.41
rs9920	7	116200092	C	T	0.09	0.79	0.14	2.60E-08	CAV1	0.81	0.70	2.48E-01	0.13
rs2072413	7	150647969	T	C	0.27	-1.68	0.11	1.30E-49	KCNH2	-1.47	0.51	3.91E-03	0.29
rs11779860	8	98850330	C	T	0.47	-0.61	0.10	1.70E-10	LAPTM4B	-1.14	0.47	1.53E-02	0.59
rs1961102	8	103932845	T	C	0.33	0.57	0.10	3.40E-09	AZIN1	0.02	0.57	9.66E-01	0.22
rs2485376	10	104050006	A	G	0.39	-0.56	0.10	2.70E-08	GBF1	-1.27	0.47	6.49E-03	0.42
rs800338	11	2473456	A	G	0.84	2.29	0.27	1.33E-17	KCNQ1	1.76	0.74	1.70E-02	0.94
rs2074238	11	2484803	C	T	0.92	5.41	0.49	7.34E-28	KCNQ1	4.57	1.42	1.27E-03	0.97
rs7122937	11	2486550	T	C	0.19	1.93	0.12	1.20E-54	KCNQ1	0.68	0.50	1.75E-01	0.31
rs174583	11	61609750	T	C	0.34	-0.57	0.09	8.20E-11	FADS2	-0.03	0.53	9.59E-01	0.25

rs3026445	12	110723203	C	T	0.36	0.62	0.09	2.90E-12	ATP2A2	0.34	0.48	4.79E-01	0.35
rs728926	13	74513122	T	C	0.36	0.57	0.10	2.10E-08	KLF12	1.56	0.52	2.97E-03	0.29
rs2273905	14	102974999	T	C	0.36	0.61	0.09	4.00E-11	ANKRD9	-1.25	0.47	7.32E-03	0.45
rs3105593	15	50845018	T	C	0.45	0.66	0.10	3.10E-12	USP50-TRPM7	0.71	0.47	1.31E-01	0.53
rs1296720	16	3873642	C	A	0.20	0.83	0.13	3.60E-10	CREBBP	0.16	0.57	7.82E-01	0.20
rs735951	16	11693536	A	G	0.46	-1.15	0.10	2.30E-28	LITAF	-1.21	0.46	8.93E-03	0.49
rs246185	16	14395432	C	T	0.34	0.72	0.10	2.60E-13	MKL2	0.52	0.52	3.15E-01	0.29
rs246196	16	58574253	C	T	0.26	-1.73	0.11	2.00E-57	CNOT1	-1.89	0.53	3.95E-04	0.25
rs1052536	17	33331575	C	T	0.53	0.98	0.10	6.20E-25	LIG3	-0.74	0.47	1.13E-01	0.50
rs4793397	17	68520389	T	C	0.46	-1.18	0.19	2.87E-10	KCNJ2	-0.75	0.47	1.12E-01	0.48

*Included SNPs were genome-wide significant ($p < 5 \times 10E-08$) in two large meta-analysis of QTc (18, 20).

Supplemental Table 4. Main effects and interaction analyses of 4 SNPs reported previously to modify the effects of known *KCNQ1* pathogenic variants on QTc.

	MAF	MAF Amish/Eur.	Effect size on QTc (ms)	Main effect p-value	Interaction with *KCNQ1* p.T224M, p-value	Reference
NOS1AP rs10494366	0.537	0.841	-2.69	3.11E-09	7.59E-01	Post et al., 2007
NOS1AP rs1415262	0.576	0.904	-2.59	1.89E-08	7.34E-01	Post et al., 2007
KCNH2 rs1805123	0.265	1.045	-1.98	6.57E-05	1.39E-01	Kapplinger et al., 2017
AKAP9 rs11772585	0.141	1.599	-0.22	7.22E-01	3.44E-01	Villiers et al., 2014

Post et al., Associations between Genetic Variants in the NOS1AP (CAPON) Gene and cardiac repolarization in the Old Order Amish. Hum Hered. 2007; 64: 214–219.

Kapplinger et al., *KCNQ1* p. L353L affects splicing and modifies the phenotype in a founder population with long QT syndrome type 1. J Med Genet 2017; 54, 390-398.

De Villiers et al., *AKAP9* is a genetic modifier of congenital long-QT syndrome type 1. Circulation: Cardiovasc Genet 2014; 7: 599-606.

Supplemental Table 5. Mean and maximal QTc by sex for carriers (Panel A) and noncarriers (Panel B).

A. Carriers

	***KCNQ1* carriers total**	**Female (F)**	**Male (M)**	**p-value F vs M (age adj.)**
N	88	49 (56%)	39 (44%)	
Age (years)	46.4 ± 17.0	45.1 ± 18.6	48.2 ± 14.8	
Mean QTc Normal supine*, n/total (%) Supine ± SD, ms Standing ± SD, ms	 40/88 (46%) 460 ± 29 483 ± 40	 23/49 (47%) 461.5 ± 22.4 486.2 ± 37.4	 17/39 (43.6%) 458.4 ± 35 479.2 ± 43	 0.51 0.54
Maximal QTc Normal supine*, n/total (%) Supine ± SD, ms Standing ± SD, ms	 17/71 (17%) 480 ± 32 502 ± 43	 10/49 (20.4%) 482.1 ± 27.9 503.9 ± 36.6	 7/39 (18%) 475.5 ± 37.2 498.9 ± 50.5	 0.30 0.12

* Normal for males < 450 milliseconds (ms) and for females <460 ms; SD = standard deviation.

B. Non-carriers

	***KCNQ1* non-carriers total**	**Female (F)**	**Male (M)**	**p-value F vs M (age adj.)**
N	54	25 (46%)	29 (54%)	
Age (years)	45.02 ± 16.7	43.4 ± 17.3	46.4± 16.4	

Mean QTc Normal supine*, n/total (%) Supine ± SD, ms Standing ± SD, ms	50/54 (92.6%) 421.7 ± 23.2 435.3 ± 25.8	23/25 (92%) 433.5 ± 21 449.5 ± 20.3	27/29 (93%) 411.6 ± 20.2 423.6 ± 24.3	0.0003 0.0001
Maximal QTc Normal supine*, n/total (%) Supine ± SD, ms Standing ± SD, ms	44/54 (81.5%) 434.9 ± 26.1 453.4 ± 33.8	19/25 (76%) 447.7 ± 26.3 465.3 ± 20.8	25/29 (86%) 423.9 ± 20.6 443.6 ± 39.3	0.0005 0.018

* Normal for males < 450 milliseconds (ms) and for females <460 ms; SD = standard deviation.

Supplemental Table 6. Supporting evidence for variant interpretation.

Criterion	Criterion Description	Source and Interpretation
	Initial Classification Evidence: VUS	
PM2_Supporting	Observed with extremely low frequency in the Genome Aggregation Consortium (gnomAD) database.	MAF 0.000008890 (1 allele) in the European (non-Finnish) subpopulation of gnomAD. Overall gnomAD MAF 0.00000423.
PP3	Multiple lines of computational evidence	See Supplemental Table 7.
PM1	Location within a transmembrane region of the protein that has minimal benign variation among individuals in population databases and in the literature.	PMID: 27761162, 17227916; ClinVar does not include any benign or likely benign variants in this region.
Miscellaneous	Identified in one patient.	PMID: 19716085
	Reclassification with Addition of Amish Data: Likely Pathogenic	
PS4_Moderate	The prevalence of the variant in affected In individuals is significantly increased with an with the prevalence in controls. >450	dataset of 5521 individuals, compared 9.8% of subjects abnormal QTc, defined as for men and >460 for women (9.8%), were heterozygous for the *KCNQ1* variant compared to 1.7% of those with a normal QTc (1.7%) (p < 1E-7). We also identified clinical evidence of LQTS (QTc ≥500 ms and/or Schwartz score of ≥3.5) in 34/88 (38.6%) of carriers vs. 3/54 (5.5%) of non-carriers (p=0.0006).

	Reclassification with Addition of Functional Data: Pathogenic	
PS3	Functional studies show abnormal protein function.	*In vitro* studies show the p.Thr224Met variant has a loss of cardiac IKs function.

Supplemental Table 7. Computational predictor pathogenicity scores for p.T224M variant (taken from Varsome 3/26/20).

	Prediction	Score	Converted rankscore (1) or Rankscore (2)
MutationTaster*	Disease causing	0.9999 (Accuracy)	0.5876 (1)
FATHMM	Damaging	-4.96	0.9845 (1)
LRT*	Deleterious	0	0.8433 (1)
SIFT*	Damaging	0	0.9125 (1)
PROVEAN	Damaging	-5.09, -4.59, -4.67	0.8296 (1)
REVEL*	Pathogenic	0.948	0.9901 (2)
MetaSVM*	Damaging	1.0902	0.9925 (2)
MetaLR*	Damaging	0.9385	0.9798 (2)
GERP v2010	Conserved	4.32	n/a

*Based on dbNSFP version 4.0

Supplemental Table 8. *KCNQ1* exon 4 variants in gnomAD (attached separately as Excel file)

Supplemental Table 9. *KCNQ1* exon4 variants in ClinVar (attached separately as Excel file)

D. References for supplemental materials

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