ORIGINAL RESEARCH

Recurrent Pregnancy Loss and Concealed Long-QT Syndrome

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BACKGROUND: Recurrent pregnancy loss affects 1% to 2% of couples attempting childbirth. A large fraction of all cases remains idiopathic, which warrants research into monogenic causes of this distressing disorder.

METHODS AND RESULTS: We investigated a nonconsanguineous Estonian family who had experienced 5 live births, intersected by 3 early pregnancy losses, and 6 fetal deaths, 3 of which occurred during the second trimester. No fetal malformations were described at the autopsies performed in 3 of 6 cases of fetal death. Parental and fetal chromosomal abnormalities (including submicroscopic) and maternal risk factors were excluded. Material for genetic testing was available from 4 miscarried cases (gestational weeks 11, 14, 17, and 18). Exome sequencing in 3 pregnancy losses and the mother identified no rare variants explicitly shared by the miscarried conceptuses. However, the mother and 2 pregnancy losses carried a heterozygous nonsynonymous variant, resulting in p.Val173Asp (*rs199472695*) in the ion channel gene *KCNQ1*. It is expressed not only in heart, where mutations cause type 1 long-QT syndrome, but also in other tissues, including uterus. The p.Val173Asp variant has been previously identified in a patient with type 1 long-QT syndrome, but not reported in the Genome Aggregation Database. With heterologous expression in CHO cells, our in vitro electrophysiologic studies indicated that the mutant slowly activating voltage-gated K+ channel (I_{Ks}) is dysfunctional. It showed reduced total activating and deactivating currents ($P<0.01$), with dramatically positive shift of voltage dependence of activation by ≈10 mV ($P<0.05$).

CONCLUSIONS: The current study uncovered concealed maternal type 1 long-QT syndrome as a potential novel cause behind recurrent fetal loss.

Key Words: exome ■ *KCNQ1* ■ long-QT syndrome ■ miscarriage ■ recurrent pregnancy loss

R ecurrent pregnancy loss (RPL) is a disease defined by the spontaneous demise of ≥2 pregnancies, affecting ≈1% to 2% of women.[1,2] RPL has a long list of different causes, as both maternal and fetal, as well as combined factors, may be responsible. As a major known cause, up to 50% of products of conception of patients with RPL have gross genomic rearrangements.[3] However, it is estimated that roughly half of RPL cases remain truly idiopathic. Because the familial history of miscarriage increases the risk to RPL ≈2-fold,[4] it is likely that some of these cases have unknown monogenic origins.[3,5] In addition to pregnancy loss being an obvious negative life event, there are emerging data that these women are at an increased

risk of various health problems later in life. Reported comorbidities include type 2 diabetes mellitus,[6] autoimmune and cardiovascular complications, such as atherosclerosis, cerebral infarction, heart failure, and pulmonary embolism, as well as psychiatric diseases.[7-9] These observations make it especially important to identify the underlying genetic factors with pleiotropic effects that may predispose to pregnancy failure as well as to the long-term health of the woman. Until now, monogenic causes of RPL remain largely unexplored, and only a handful of studies using exome sequencing have been published.[10]

The current study aimed to clarify the potential genetic cause of idiopathic RPL in an Estonian couple

CLINICAL PERSPECTIVE

What Is New?
- The current study uncovered concealed maternal type 1 long-QT syndrome as a potential novel cause behind recurrent pregnancy loss.
- In vitro electrophysiological studies showed that the *KCNQ1* p.V173D (c.518T>A) variant causes dysfunction and marked attenuation of the I_{Ks} channel.

What Are the Clinical Implications?
- This novel finding emphasizes the value of personalized medicine and the importance of in-depth assessment of all potentially causative genotype-to-phenotype links.
- Genotype-positive, phenotype-negative subjects should avoid exposure to any exogenous risk factors, such as competitive sports or QT-prolonging drugs.
- Early detection of *KCNQ1* pathogenic variant carriers by testing among family members facilitates timely monitoring, treatment, and prevention of cardiac arrhythmias; clinical assessment of unexplained recurrent fetal losses should consider concealed maternal and/or fetal long-QT syndrome as a possible cause.

Nonstandard Abbreviations and Acronyms

LQTS1	type 1 long-QT syndrome
PSAP	population sampling probability
QTcB	the heart-rate corrected QT interval based on the Bazett's formula
QTc	QT interval corrected for the heart rate
RPL	recurrent pregnancy loss
WES	whole-exome sequencing

who had experienced in total 9 pregnancy losses, 3 early miscarriages and 6 fetal deaths after 10 weeks' gestation.

METHODS

The authors declare that all supporting data are available within the article and its online Supplemental Material.

Ethical Approval

The study was approved by the Ethics Review Committee of Human Research of the University of Tartu, Tartu, Estonia (permission Nos. 117/9, 16.06.2003; 146/18, 27.02.2006; 150/33, 19.06.2006; 212/M-32, 09.03.2012; and 286/M-18, 15.10.2018) and was performed in compliance with the Declaration of Helsinki. A written informed consent to participate in the study was acquired from all adult research participants before recruitment.

Exome Sequencing and Variant Detection

DNA samples from the proband's blood and 3 miscarriage events (III-5, III-9, and III-13; Figure 1A) were subjected to whole-exome sequencing (WES). The WES data generation and data analysis were performed as described in the study by Kasak et al.[11] Briefly, wet-laboratory processing, base calling of the raw sequencing data, primary sequence analysis, and variant calling were performed at FIMM (Institute for Molecular Medicine Finland) Next Generation Sequencing Service (Helsinki, Finland). Whole exome enrichment was undertaken with the SeqCap EZ MedExome Target Enrichment Kit (Roche NimbleGen, Madison, WI) following the manufacturer's protocol. Sequencing was performed on Illumina HiSeq 2500 sequencing system (San Diego, CA). Primary sequence analysis and variant calling were performed using the Variant Calling Pipeline (VCP3.7).[12] Illumina paired-end reads were trimmed with Trimmomatic (version 0.36) and aligned against human genome build hg19 with the Burrows-Wheeler Aligner (version 0.6.2).[13] Next, polymerase chain reaction duplicates were removed with Picard MarkDuplicates (version 2.9.0). Single-nucleotide variants and insertions/deletions were called with SAMtools (version 1.4)[14] and Pindel (version 0.2.5b8),[15] respectively. Mean target coverage ranged from 91× to 122×, with an average of 97% of bases covered at 20×.

Exome Data Filtering and Prioritization of Variants

Variant call format (VCF) files of the pregnancy loss samples were annotated using ANNOVAR (version 20191024).[16] Among all coding variants (exonic and splicing), synonymous and common variants with minor allele frequency of >0.1% in Genome Aggregation Database[17] and Exome Aggregation Consortium[18] were removed (Figure S1). Only shared variants in all pregnancy loss samples were retained for the analysis.

To prioritize variants from the WES data of the proband, the population sampling probability (PSAP) pipeline[19] developed for n-of-1 analyses was applied. It is a model-based framework to evaluate the significance of genotypes ascertained from a single case by determining the by-chance probability of sampling the detected genotypes in the unaffected population based on the pathogenicity scores and observed frequencies of variants. The variants were prioritized to
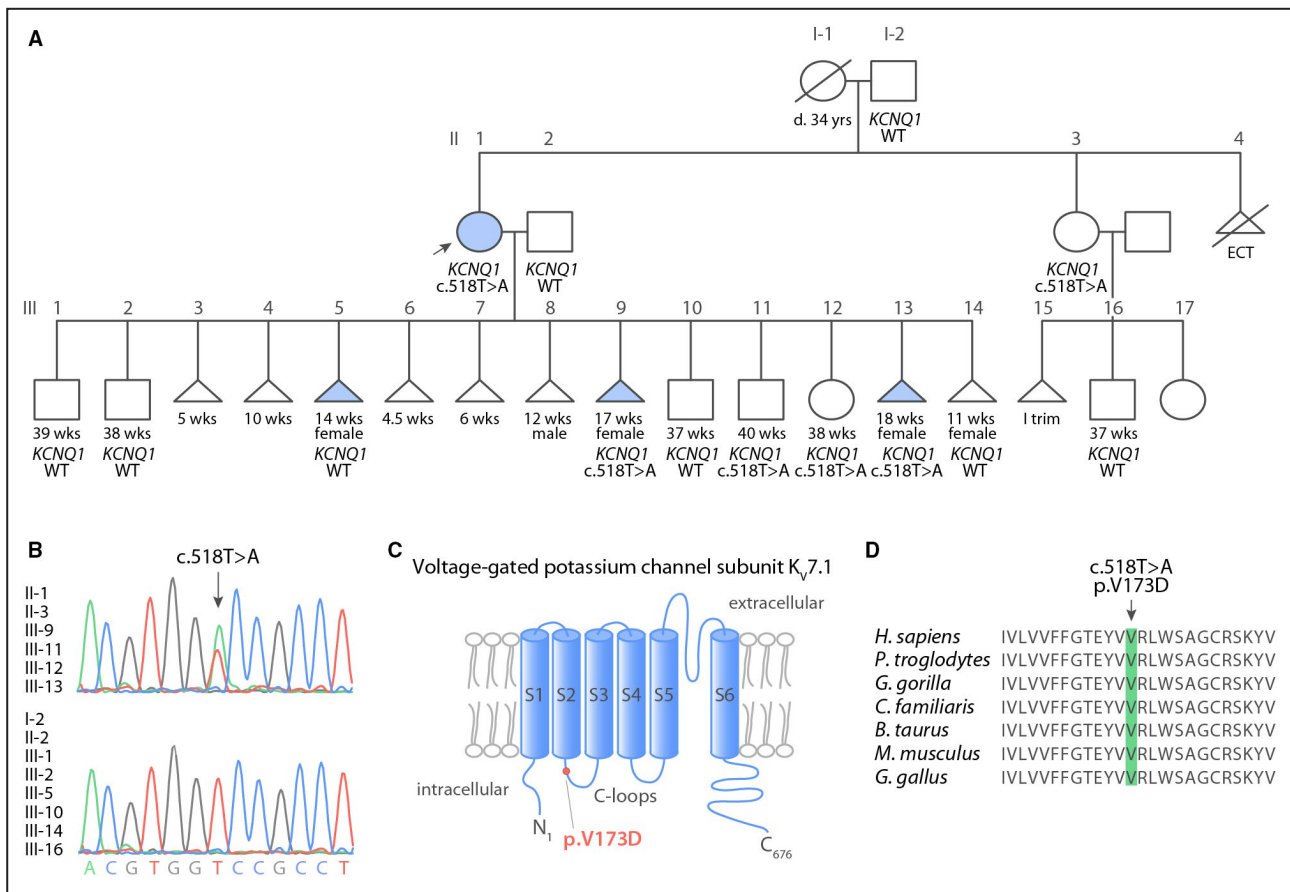
Figure 1. Identification of the *KCNQ1* variant.
A, Pedigree of the family. Circles denote female family members, squares denote male members, and triangles indicate spontaneous pregnancy losses. The proband is indicated with an arrow. Solid symbols indicate pedigree members, whose genomic DNA was subjected to exome sequencing. **B**, Sanger sequencing confirmation of the *KCNQ1* p.V173D variant (*rs199472695*) in the family. **C**, Molecular position of the $K_V7.1$ variant p.V173D in the first C-loop. **D**, The conservation of the amino acid residue affected by the *KCNQ1* variant in different species. d, death; ECT indicates ectopic pregnancy; trim, trimester; wks, gestational weeks; WT, wild type and yrs, years.

satisfy the following criteria: (1) missense and loss-of-function variants; (2) low minor allele frequency (≤0.001 Exome Aggregation Consortium, Genome Aggregation Database, and 1000 Genomes Project) or a previously undescribed variant; and (3) the PSAP statistical significance value ≤0.005 and the Combined Annotation-Dependent Depletion score ≥20. PSAP pipeline was also applied for the extended assessment of individual fetal exomes.

Next, for both parts of the analysis (pregnancy loss samples and the proband), in-house blacklisted variants were removed. Visual inspection of the quality of sequencing reads was performed using the Integrative Genomics Viewer[20] software for all prioritized rare and novel variants to ensure high quality of retained variants. All the retained variants were manually inspected using scientific literature and genome databases. Variants were classified according to the American College of Medical Genetics and Genomics guidelines.[21]

KCNQ1 p.V173D Variant Validation and Extended Family Testing by Sanger Sequencing

Primers (forward, TGCCTATGGACATGAGCTGA; reverse, GGGAATCTGTGAGGGACCAA; sequencing, GCATGGCTGGGTTCAAACA) for amplification and sequencing of the *KCNQ1* p.V173D variant (*rs199472695*) were designed in Primer3web (https://bioinfo.ut.ee/primer3/), tested by National Center for Biotechnology Information Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and GenomeTester (https://bioinfo.ut.ee/genometester/). DNA fragments (individuals I-2, II-1, II-2, II-3, III-1, III-2, III-5, III-9, III-10, III-11, III-12, III-13, III-14, and III-16) were amplified by standard polymerase chain reaction, sequenced with the BigDye Terminator v.3.1 Cycle Sequencing Kit, and run on an ABI 3730 DNA Analyzer (both Applied Biosystems, Carlsbad, CA). Sequences were analyzed with 4Peaks (https://nucleobytes.com/4peaks/index.html).

Functional Assessment of the *KCNQ1* p.V173D Variant
Site-Directed Mutagenesis

The p.V173D 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*:internal ribosome entry site (IRES):green fluorescent protein (GFP), and the wild-type and mutant sequences were confirmed. Plasmids were transfected into CHO cells using Fugene 6 (Promega) following manufacturer's instructions. Wild-type or p.V173D pIRES2-GFP *KCNQ1* plasmids were cotransfected in equimolar ratios with a pIRES2-dsRed expression plasmid expressing wild-type *KCNE1* (the I_{Ks} accessory subunit). Two days after transfection, cells expressing both *KCNQ1* in green and *KCNE1* in red were selected with fluorescent lights for electrophysiologic functional studies.

Electrophysiologic Functional Studies

Whole-cell voltage clamp experiments were performed at room temperature (22 °C–23 °C) using a patch-clamp system: MultiClamp 700B amplifier, 1350 DigiData, and data acquisition software pClamp 10.7 (Molecular Devices Inc, Sunnydale, CA). Patch glass microelectrodes with 3 to 5 mΩ were used to patch cells. The pipette (intracellular) solution contained (in mmol/L): KCl 110, MgCl$_2$ 1.0, ATP-K2 5.0, 1,2,-bis(o-aminophenoxy)ethane-N,N,-N0,N0-tetra-acetic acid (BAPTA)-K4 5.0, and HEPES 10, with the pH of 7.2, adjusted with KOH. The extracellular solution contained (in mmol/L): NaCl 145, KCl 4.0, MgCl$_2$ 1.0, CaCl$_2$ 1.8, glucose 10, and HEPES 10, with the pH of 7.4, adjusted with NaOH. Data acquisition was performed using pClamp 10.7, sampling at 1 kHz, and low-pass filtered at 5 kHz. Activating current was elicited with 5-s depolarizing pulses from a holding potential of −80 to 80 mV at 20-mV increments, and deactivating tail current was recorded on return to −40 mV. The voltage-clamp protocol is shown in Figure 2A. Pulses were delivered every 30 s. I-V relationships were analyzed by fitting the Boltzmann equation to the data: $I=I_{max}/\{1+\exp[(V_t–V_{1/2})/k]\}$, where I_{max} is the maximal current, V_t is the test potential, $V_{1/2}$ is the membrane potential at which 50% of the channels are activated, and k is the slope factor. Current densities in picoamperes per picofarad (pA/pF) were obtained after normalization to cell surface area calculated by the Membrane Test in pClamp 10.7.

Statistical Analysis

Functional I_{Ks} analysis data are expressed as mean±SEM. For comparisons of 2 groups of data, 1-way repeated-measures ANOVA incorporated in the Ultimate Software for Analysis & Graphing (OriginPro version 8.5.1; OriginLab Corp, Northampton, MA) was used to analyze the I_{Ks} densities at individual testing membrane potentials. A $P<0.05$ or 0.01 is considered statistically significant.

RESULTS

Clinical Case

We investigated a nonconsanguineous Estonian family who had experienced 5 live births, intersected by 3 early pregnancy losses and 6 fetal deaths (Figure 1A and Table 1). Three miscarriages occurred in the second trimester, at 14+5, 17+0, and 18+0 gestational weeks. Detailed health evaluation of the couple for known clinical risk factors of RPL was performed after the third miscarriage (maternal and paternal age at assessment, 26 and 32 years, respectively). Both partners had a normal karyotype and negative repeated test results for genital tract infections (urogenital chlamydia, gonorrhea, ureaplasmosis, and mycoplasmosis). The proband (subject II-1, referring to second generation, first case; Figure 1A) has a normal regular menstrual cycle (27–29 days) and no major uterine anomalies (based on repeated ultrasonography and hysterosonogram) and endocrinological disorders (diabetes mellitus, hypothyroidism/hyperthyroidism, hyperprolactinemia, and hyperhomocysteinemia; Table S1). Maternal antiphospholipid syndrome (anticardiolipin antibodies, β-2 glycoprotein 1 antibodies, and lupus anticoagulants) and risk variants predisposing to thrombophilia, factor V Leiden (MIM: 612309, *F5* p.R534Q, *rs6025*), and factor II prothrombin deficiency (MIM: 176930, *F2* c.G20210A, *rs1799963*) were excluded.

Autopsies, performed in 4 of 6 fetal death cases, were normal; and all these fetuses had a normal karyotype. Also, screening of pathogenic submicroscopic chromosomal variants did not result in any clinically relevant findings for either of the partners or 2 miscarried products of conception included into this analysis (cases III-5 and III-8; Figure 1A).[22,23] Available pathohistological reports for the placental tissues of 3 late miscarriages (14–18 gestational weeks) excluded placental inflammation as a contributing factor, but signs of focal delayed placental maturation and circulatory complications were reported (Table 1). Four live-born sons of the couple have no congenital malformations (Table 2). However, their daughter (subject III-12) was diagnosed at the age of 3 years with congenital ventricular septal
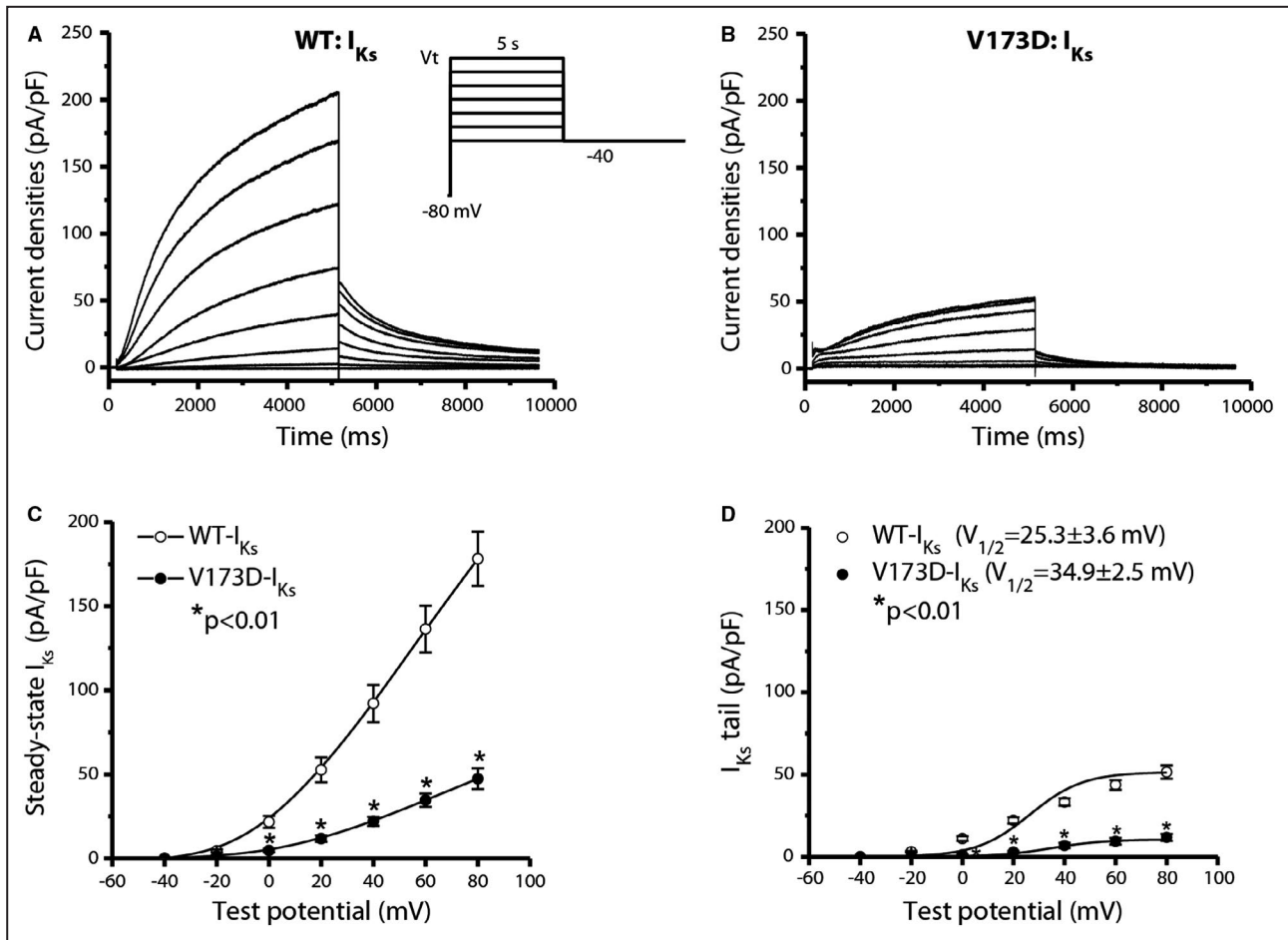
Figure 2. *KCNQ1* mutation p.V173D caused a loss of cardiac I_{Ks} function.
A and **B**, Typical I_{Ks} traces recorded in CHO cells in which either wild-type (WT) *KCNQ1* or V173D were coexpressed with WT KCNE1. **C** and **D**, I_{Ks} steady-state and tail current densities in the 2 groups of cells (n=10 each). The mutant channel p.V173D significantly reduced total I_{Ks} steady-state and tail currents with a dramatic positive shift of the voltage dependence of activation, by ≈10 mV (*P*<0.05). Current densities were expressed in pA/pF after normalization of current amplitude to cell capacitance. The voltage clamp protocol is shown in the insert.

defect (2-degree murmur) with spontaneous closure by the age of 7 years (Data S1).

Genetic Analysis of the Proband and 3 Miscarried Fetuses

The pedigree structure suggested a possible genetic predisposition to RPL on the maternal side of the family as early pregnancy complications had been reported also for the proband's mother (I-1; ectopic pregnancy) and sister (II-3; miscarriage) (Figure 1A). An alternative scenario to explain the extensive series of pregnancy losses was pathogenic combinations of maternal and paternal rare recessive variants in all miscarried conceptuses. We performed WES using genomic DNA extracted from the maternal blood and 3 available miscarried products of conception (Figure 1A). No pathogenic variants were identified in any of these samples in genes previously reported in WES studies of RPL

cases.[10,24] In the joint assessment of the WES data sets of 3 aborted fetuses, also no shared homozygous or biallelic rare disease-causing coding or splicing variants were detected (Figure S1 and Table S2). For the analysis of the proband's WES data set, we applied the PSAP pipeline that has been specifically developed for the filtering and pathogenic variant prioritization of n-of-1 exome data sets.[11,19] When considering only rare variants (minor allele frequency, <0.001) with high scores of possible functional effect (Combined Annotation-Dependent Depletion score ≥20[25]) and PSAP statistical significance threshold *P*≤0.005, most retained variants in the proband were recessive and classified on the basis of the American College of Medical Genetics and Genomics guidelines[21] as variants of uncertain significance. A single likely pathogenic variant was identified: an ultrarare missense heterozygous change c.518T>A (p.V173D, *rs199472695*) in the *KCNQ1* gene (MIM: 607542) (Figure 1B and Table S3).

Table 1. Recurrent Pregnancy Loss History of the Proband

Pregnancy loss identifier	Maternal age, y	Gestational age	Karyotype	Pregnancy data	Fetal malformations	Placental phenotype	Microdeletions/duplications*	KCNQ1 c.518T>A
III-3	25	5 wk	NA	Empty sac pregnancy loss	NA	NA	NA	NA
III-4	25	10 wk, 5 d	NA	Fetal death	NA	NA	NA	NA
III-5	26	14 wk, 5 d	46,XX	Vanishing twin at 6–7 wk, fetal death at 14 wk+5 d	Not detected	Focal delayed maturation and circulatory disorders, no inflammation	Not detected	TT (WT)
III-6	26	4.5 wk	NA	Biochemical pregnancy loss	NA	NA	NA	NA
III-7	26	6 wk	NA	Yolk sac pregnancy loss	NA	NA	NA	NA
III-8	27	12 wk	46,XY	Fetal death	NA	NA	Not detected	NA
III-9	28	17 wk	46,XX	Fetal death	Not detected	Focal thrombotic vasculopathy, abundant erythroblasts in placental vessels, may indicate fetal anemia, no inflammation	NA	TA
III-13	39	18 wk	46,XX	Fetal death	Left hand mild clinodactyly, low-lying ears, asymmetric fetal growth restriction	Hypercoiling of umbilical cord (coiling index, 0.61), normal finding with some postmortem changes: minimal villous fibrosis, no inflammation	NA	TA
III-14	40	11 wk, 3 d	46,XX	Fetal death	Not detected	NA	NA	TT (WT)

NA indicates not assessed; and WT, wild type.

*On the basis of the analysis using chromosomal microarray.

In applying the same variant filtering pipeline for the individual fetal exomes, 2 of the 3 analyzed pregnancy loss cases (III-9 and III-13) also carried the *KCNQ1* p.V173D substitution. No additional (likely) pathogenic variants were identified in fetal exomes (Table S4).

The *KCNQ1* p.V173D variant is absent from all population-based human genetics public databases, but is in ClinVar (accession identifier: VCV000053057). It was reported in one individual diagnosed with type 1 long-QT syndrome (LQTS1; MIM: 192500),[26] characterized by a prolonged QT interval in the ECG. This represents a high risk to syncope or sudden death from cardiac arrhythmia.[27] LQTS1 condition is inherited in an autosomal dominant manner with reduced (30%–40%)[28] penetrance and variable expressivity between as well as within families.[29,30] More recently, channelopathy genes have also been considered as relevant candidates for unexplained stillbirth and pregnancy losses without fetal anomalies.[31-33] In the index family, the *KCNQ1* p.V173D variant was also identified not only in 2 of the 3 analyzed pregnancy losses (III-9 and III-13), but also in 2 live-born children of the couple (III-11 and III-12) and the sister of the proband (II-3; Figure 1A and 1B).

Cardiological Assessment of the *KCNQ1* p.V173D Variant Carriers

In retrospective assessment of clinical data gathered during the monitored pregnancies of the proband, the only documented symptoms possibly indicating a cardiovascular phenotype were at 17 to 18 weeks of pregnancy III-13. The patient presented transient hypertension, swelling of feet-hands, palpitations, and sweating at night for a couple of weeks, and she was administered labetalol (nonselective β-blocker and selective α1-blocker). The symptoms were interpreted as psychosomatic reaction to fetal loss.

After the genetic diagnosis, follow-up clinical interviews and monitoring of cardiac function were performed for the family members carrying the pathogenic *KCNQ1* p.V173D variant: the proband, her sister, and 2 children. During the cardiac health assessment, the proband (aged 41 years) and her sister (II-3; aged 38 years; diagnosed with chronic magnesium deficiency) were taking magnesium and potassium supplements. Both sisters have reported occasional palpitations, faintness, and presyncope during physical or emotional stress. The proband's ECG showed normal sinus rhythm (heart rate, 84 beats per minute; electrical axis, 62°) and no abnormal repolarization and depolarization, but a borderline QT prolongation (the heart-rate corrected QT interval based on the Bazett's formula [QTcB], 475 ms, compared with the threshold of normal QTcB <450 ms in women;[34] Data S1). In addition, the proband was assigned to a 24-hour Holter monitoring

Table 2. Clinical Characteristics of the Proband's Live-Born Children

Live birth				Newborn		Childhood		Genetics
Identifier	Maternal age, y	Gestational week	Pregnancy	Sex	Weight, g*	Health problems	QTcB, ms	*KCNQ1* c.518T>__A__
III-1	20	39	Uncomplicated	Boy	3300	Atopic dermatitis, asthma, minor scoliosis		TT (WT)
III-2	24	38	Uncomplicated	Boy	3600	Minor sport injuries		TT (WT)
III-10	29	37	Administration of enoxaparin†	Boy	3364	Atopic dermatitis, umbilical hernia (surgery at the age of 8 y)		TT (WT)
III-11	30	40	Administration of enoxaparin†	Boy	3395	Appendicitis (surgery at the age of 4 y), minor sport injuries	442/438 (11 y)‡	T__A__
III-12	33	38	Administration of enoxaparin†	Girl	3344	Napkin dermatitis, congenital ventricular septal defect (asymptomatic, detected accidentally by auscultation at the age of 3 y [2-degree murmur], spontaneous closure by the age of 7 y)	420/428 (7/8 y)§	T__A__

WT indicates wild type; and QTcB, corrected QT interval estimated using the Bazett formula.[34]

*All normal vaginal deliveries.

†Because of focal delayed maturation of villi and circulatory disorders for pregnancy loss case at 14 weeks 5 days (III-5) and focal thrombotic vasculopathy for pregnancy loss at 17 weeks (III-9), low-molecular-weight heparin enoxaparin as a preventive management measure of recurrent abortions was administrated during subsequent pregnancies.

‡Lying/sitting position.

§Two assessments at the ages of 7 and 8 years.

that also revealed prolonged QT interval corrected for the heart rate (QTc) at nighttime. The longest QT interval corrected for the heart rate at night was measured 534 ms, whereas the longest daytime QT interval corrected for the heart rate was 508 ms (including daily exercises; Data S1). Currently, the patient has been referred to a thorough cardiological workup. To date, no ECG pathological feature has been detected for the proband's sister (detailed report unavailable because of residency abroad).

The proband's daughter (III-12) was diagnosed at the age of 3 years with a ventricular septal defect that was confirmed to have closed on its own by the age of 7 years at the follow-up cardiology visit. She has complained of occasional chest pain at resting state, occurring more frequently during school period. Although she had normal ECG (aged <15 years: reference threshold of normal QTcB <440 ms), a minor sinus arrhythmia was detected (heart rate, 68 beats per minute; axis, 57°; PR, 133 ms; QRS, 69 ms; QTcB, 428 ms; normal depolarization and repolarization; Data S1). Therefore, a follow-up cardiac assessment in 5 years was suggested by the managing pediatric cardiologist. The proband's youngest son, III-11 (aged 11 years), received a pediatric cardiologist's assessment after the genetic diagnosis of LQTS1. Also in his case, the ECG was normal, but in lying position a minor sinus arrhythmia was reported (heart rate, 66 beats per minute; axis, 79°; PR, 120 ms; QRS, 82 ms; QTcB, 442 ms; normal depolarization and repolarization; standing: heart rate, 71 beats per minute; QTcB, 438 ms) and a follow-up visit was scheduled in 1 year.

In Vitro Functional Effect of the *KCNQ1* p.V173D Variant

To establish whether the *KCNQ1* p.V173D variant confers identifiable functional defects, we performed in vitro electrophysiological studies using the whole cell patch-clamp technique. The *KCNQ1* variant p.V173D was engineered in a recombinant $K_V7.1$ potassium channel plasmid vector and heterologously coexpressed with *KCNE1* cDNA in CHO cells to assess its functional consequence (see Methods for details). Compared with the wild-type channel (Figure 2), the mutant channel p.V173D statistically significantly reduced total activating and deactivating currents ($P<0.01$), with dramatically positive shift of voltage dependence of activation by ≈10 mV ($P<0.05$). Thus, $K_V7.1$-V173D causes a loss of function and marked attenuation of I_{Ks}, classifying *KCNQ1* p.V173D (c.518T>A) as a pathogenic variant. Furthermore, we examined the responses of wild type–I_{Ks} and the *KCNQ1* variant p.V173D-I_{Ks} to β-adrenergic receptor agonist isoproterenol (1 µmol/L). As shown in Figure S2, wild type–I_{Ks} was dramatically increased by isoproterenol, a well-recognized effect in agreement with previous studies.[35,36] However, the variant p.V173D-I_{Ks} was slightly enhanced by isoproterenol.

DISCUSSION

The current study uncovered concealed maternal LQTS1 as a potential novel cause behind recurrent fetal loss. Exome sequencing analysis of the

Table 3. Relevance of Maternal *KCNQ1* Mutations to the Risk of RPL

KCNQ1 function[29,40,42]
K+ homeostasis maintenance needed for electrolyte and hormone transport
KCNQ1 forms the slowly activating voltage-gated potassium ion channel I_{Ks} in the heart by coassembly with KCNE1
Depends on the tissue and available subunits (KCNE1–5) that drastically modify the channel kinetics
KCNQ1 expression in human (ProteinAtlas and Wang et al[40])
One heart-specific isoform
Other isoform ubiquitously expressed
Diseases related to loss of function in human (OMIM)
LQTS1; causes syncope and sudden death in response to exercise or emotional stress (AD or incomplete penetrance)
Familial atrial fibrillation; causes palpitations, syncope, thromboembolic stroke, and congestive heart failure (AD)
Short-QT syndrome; causes syncope and sudden death (AD)
Jervell and Lange-Nielsen syndrome; characterized by congenital deafness and prolongation of the QT interval (AR)
Relevance to pregnancy complications
Women with LQTS are at increased risk for fetal death and IUGR caused by placental or myometrial dysfunction[44]
KCNQ1 is important for contractile function of the uterine smooth muscle and vascular tone regulation in pregnancy[45,46]
Stillbirth[47]
Sudden infant death syndrome[48]
Trophoblast differentiation[49]

AD indicates autosomal dominant; AR, autosomal recessive; IUGR, intrauterine growth restriction; LQTS1, type 1 long-QT syndrome; OMIM, Online Mendelian Inheritance in Man; and RPL, recurrent pregnancy loss.

proband, who had experienced 9 pregnancy losses, identified a heterozygous nonsynonymous variant, resulting in p.V173D in the ion channel gene *KCNQ1* (rs199472695). Before, this variant has only been identified once in an individual with LQTS1.[26] *KCNQ1* encodes the $K_V7.1$ potassium channel protein that forms homodimers and heterodimers with other K+ channel proteins. Its functional role is best known in the heart, where it coassembles with KCNE1 to form the slowly activating voltage-gated K+ channel I_{Ks}[37-39] (Table 3). $K_V7.1$ consists of 6 transmembrane segments, termed as S1 to S6 (Figure 1C).[40] The residue V173 is located in the first C-loop between segments S2 and S3 and is the only naturally occurring variant at this position (Figure 1C and 1D). According to VarSite server,[41] a Val to Asp residue change has a high "disease propensity" value of 2.81. The propensities measure how much more frequently a variant is seen in diseases than in the natural variant data obtained from Genome Aggregation Database[17] (values range from 0.25 [I>V] to 3.27 [C>R]). Supporting the critical function of this $K_V7.1$ region, ClinVar (accessed March 2021) lists 38 likely pathogenic/pathogenic variants for 27 residues between amino acids 169 and 196 (S2–S3 C-loop). In

addition, $K_V7.1$ interacts with calmodulin through residues in the S2 to S3 loop. This interaction is important for channel assembly, trafficking, and channel gating.[42] The p.V173D change could affect the binding of calmodulin and thus impair channel function. The functional assessment in vitro supported the pathogenicity of the variant, leading to the dysfunctional I_{Ks} channel. Compared with the wild-type–I_{Ks} channel, the current of the variant-expressed p.V173D-I_{Ks} channel is less sensitive to β-receptor stimulator isoproterenol. The β-receptor stimulated I_{Ks} increase is associated with intracellular cAMP-Protein kinase A (PKA) mediated phosphorylation of the channel protein.[43] The mechanism underlying lower sensitivity of the V173D channel to isoproterenol remains to be studied.

In addition to the proband, the *KCNQ1* p.V173D variant was also identified in 2 of the 3 second-trimester miscarriages (III-9 and III-13), 2 live-born children (III-11 and III-12), and the sister of the proband (II-3; Figure 1A and 1B). Therefore, it can be concluded that a fetus carrying a pathogenic *KCNQ1* variant is not per se at risk to miscarriage. Consistent with our data, a retrospective study by Cuneo et al (2020), targeting 148 pregnancies of patients with LQTS, showed that maternal, not fetal, pathogenic variants in LQTS genes, especially in the *KCNQ1*, confer increased risk for fetal deaths.[44] This analysis showed that pregnancies were significantly more likely to end in miscarriage or stillbirth with maternal rather than paternal LQTS condition (24.4% versus 3.5% of gestations, respectively). Interestingly, 2 of 3 women who reported ≥2 pregnancy losses in that study also carried pathogenic variants located in one of the C-loops of the encoded protein, as the *KCNQ1* p.V173D variant described in the current report (Table 4). Important for the clinical management of pregnant women with cardiac arrhythmias, there is evidence that pathogenic variants in LQTS genes may represent a shared cause of (late) fetal deaths and stillbirths. For example, the *KCNQ1* c.1189C>T, p.R397W variant has been reported in a case with fetal intrauterine death at 16 gestational weeks,[31] as well as in a stillbirth case at 27 gestational weeks.[33] Similarly, the *KCNQ1* c.1766G>A, p.G589D variant was identified in women who have experienced ≥1 miscarriage(s) and also a stillbirth (Table 4).[44]

It has been reported that 66% of LQTS1 carriers inherited the variant from their mother and 39% (versus expected 25%) from their maternal grandmother.[50] Also in the index family, the deceased mother of the proband (I-1) was a highly likely carrier of the *KCNQ1* p.V173D variant, passing it on to both of her daughters (Figure 1A). As an important fact, she died already at the age of 34 years, shortly after the diagnosis of thyroid cancer. Apart from the hereditary conditions, thyroid cancer mortality rate in developed countries is low and strongly related to age, with the highest rate

Table 4. *KCNQ1* Genotypes Linked to Fetal Deaths

Nucleotide change*	Protein change*	*rs* number	gnomAD MAF	Location in protein	Fetal losses, n	Gestational weeks	ACMG[21]	Ref	ClinVar: reported in LQTS
Maternal long-QT genotype (fetal genotype not available)									
c.518T>A	p.V173D	*rs199472695*	NA	S2-S3 C-loop	9	10.8±5.0	LP	This study	Yes
c.551A>C	p.Y184S	*rs199473397*	NA	S2-S3 C-loop	3	10.1±3.4	Pathogenic	44	Yes
c.760G>A	p.V254M	*rs120074179*	NA	S4-S5 C-loop	2	10.1±3.4	Pathogenic	44	Yes
c.1766G>A†	p.G589D	*rs120074190*	4.96×10⁻⁵	C-terminus	3 women with 3, 1, 1‡ fetal losses, respectively	10.1±3.4	Pathogenic	44	Yes
c.1771C>T	p.R591C	*rs199473483*	3.98×10⁻⁶	C-terminus	1	10.1±3.4	LP	44	Yes
Fetal long-QT genotype in second-trimester miscarriages (maternal genotype not available)									
c.847G>A	p.A283T	NA	NA	S5-S6 linker	NA	15.7	LP	31	No
c.1189C>T§	p.R397W	*rs199472776*	1.88×10⁻⁴	C-terminus	NA	16.0	LP	31	Yes

ACMG indicates American College of Medical Genetics and Genomics; gnomAD, Genome Aggregation Database; LP, likely pathogenic; LQTS, long-QT syndrome; MAF, minor allele frequency; NA, not applicable; and Ref, literature reference.

*According to transcript NM_000218.2, ENST00000155840.

†In gnomAD, 14 carriers (8 females) among 141, 171 subjects vs 3 of 60 women with type 1 LQTS, who all had experienced fetal losses and/or stillbirths.[44]

‡One woman had experienced a miscarriage and a stillbirth.[44]

§Also reported in a stillbirth case (gestational week 27+4).[33]

in people aged ≥75 years.[51] However, certain thyroid disorders and several drugs used as supportive care therapy in patients with cancer are associated with prolonged QT interval.[52-54] It cannot be excluded that thyroid cancer or chemotherapy could have brought on an arrhythmia, causing her death at such a young age.

The occurrence of asymptomatic carriers in this family is not unexpected as 37% of *KCNQ1* mutation carriers do not have abnormal ECGs or experience any symptoms of LQTS-related arrhythmias.[28] In patients with C-loop missense mutations compared with other pathogenic variant carriers, 2-fold higher rate of life-threatening cardiac events has been reported.[55] However, although associated with a higher risk of severe cardiac events, C-loop pathogenic variant carriers have a pronounced response to β-blocker therapy.[55] This knowledge of allelic heterogeneity in response to therapy is valuable to ensure proper clinical management to prevent severe symptoms.

$K_V7.1$ K+ channel protein encoded by *KCNQ1* wears many hats by performing various cellular tasks in the inner ear, kidney, intestine, colon, thyroid, brain, as well as airways to regulate the electric activity or maintain K+ homeostasis needed for electrolyte and hormone transport (Table 3).[37,56] Thus, it is anticipated that *KCNQ1* mutations may lead to complex and pleiotropic consequences. There are limited data on the role of K+ channels and specifically on $K_V7.1$ in the normal function and physiology of female reproductive organs.

The current study data suggest that mutations affecting $K_V7.1$ function may affect placental or uterine function. Lundquist et al (2006) have shown that *KCNQ1* is expressed in the uterus together with *KCNE1a* in the relative absence of other *KCNE* genes, suggesting the possible generation of an I_{Ks} channel complex that is involved in uterine physiology.[57] Dysfunctional channel may lead to vasoconstricted uterine arteries, causing insufficient uteroplacental blood flow (Table 3).[45-49] This is consistent with the placental phenotypes of the fetal losses that displayed focal delayed maturation and circulatory disorders, thrombotic vasculopathy, as well as intrauterine growth restriction in one case (Table 1). A pathogenic variant in another LQTS gene, *KCNJ2*, has just recently been reported in a female unexplained intrauterine death with fetal thrombotic vasculopathy.[32] Future studies are needed to fully understand the contribution of proteins regulating K+ homeostasis in pregnancy success and failure.

As an additional observation, in the index family, 4 of 5 pregnancy losses with known sex were female and vice versa 4 of 5 live-born children of the proband are boys. Progesterone and testosterone are known to have protective effects against arrhythmias, attributable to vasorelaxant effect, whereas estradiol exerts a proarrhythmic effect.[58] Testosterone levels in amniotic fluid are significantly higher in male fetuses during weeks 12 and 18.[59] This has been proposed as the cause of female predominance in LQTS[50] and could also explain the sex differences in miscarriages and live-born children in this family.

In summary, the current study uncovered a potential novel cause behind RPL and fetal death. WES revealed a rare maternal pathogenic variant p.V173D in *KCNQ1* as a possible cause to explain her extensive series of miscarriages. *KCNQ1* variants have been predominantly analyzed in patients with the diagnosis of LQTS, and reproductive history is rarely if at all reported in these individuals. To date, no familial studies on the link between RPL and LQTS1 have been performed, although first-degree relatives of women with RPL have been shown to exhibit an increased risk of cardiovascular disease.[8,9,60,61] Our study emphasizes the value of personalized medicine and the importance of in-depth assessment of all potentially causative genotype-to-phenotype links. The findings of the current study have had an impact not only on the proband, but also on the long-term clinical monitoring and counseling of a large number of family members. Genotype-positive, phenotype-negative children should be watched carefully and not be exposed to any exogenous risk factors, such as competitive sports[62] or QT-prolonging drugs.[63,64] In the era of WES, early detection of pathogenic variant carriers facilitates timely monitoring, treatment, and prevention of serious cardiac arrhythmias and, in extreme cases, sudden death. Globally, identification of novel genetic determinants of RPL reduces the number of idiopathic cases and is expected to have major individual patient impact with respect to counseling and treatment.

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Disclosures

Sources of Funding

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REFERENCES

1. Practice Committee of the American Society for Reproductive Medicine. Electronic address asrm@asrm.org. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil Steril*. 2020;113:533–535.
2. Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, Nelen W, Peramo B, Quenby S, Vermeulen N, et al. Eshre guideline: recurrent pregnancy loss. *Hum Reprod Open*. 2018;2018:hoy004. DOI: 10.1093/hropen/hoy004.
3. Kasak L, Rull K, Laan M. Genetics and genomics of recurrent pregnancy loss. In: Leung P, Qiao J, eds. *Human Reproductive and Prenatal Genetics*. 1st ed. Cambridge, MA: Academic Press Inc; 2019:463–494. DOI: 10.1016/B978-0-12-813570-9.00021-8.
4. Woolner AMF, Nagdeve P, Raja EA, Bhattacharya S, Bhattacharya S. Family history and risk of miscarriage: a systematic review and meta-analysis of observational studies. *Acta Obstet Gynecol Scand*. 2020;99:1584–1594. DOI: 10.1111/aogs.13940.
5. Rull K, Nagirnaja L, Laan M. Genetics of recurrent miscarriage: challenges, current knowledge, future directions. *Front Genet*. 2012;3:34. DOI: 10.3389/fgene.2012.00034.
6. Egerup P, Mikkelsen AP, Kolte AM, Westergaard D, Rasmussen S, Knop FK, Lidegaard O, Nielsen HS. Pregnancy loss is associated with type 2 diabetes: a nationwide case-control study. *Diabetologia*. 2020;63:1521–1529. DOI: 10.1007/s00125-020-05154-z.
7. Westergaard D, Nielsen AP, Mortensen LH, Nielsen HS, Brunak S. Phenome-wide analysis of short- and long-run disease incidence following recurrent pregnancy loss using data from a 39-year period. *J Am Heart Assoc*. 2020;9:e015069. DOI: 10.1161/JAHA.119.015069.
8. Oliver-Williams CT, Heydon EE, Smith GC, Wood AM. Miscarriage and future maternal cardiovascular disease: a systematic review and meta-analysis. *Heart*. 2013;99:1636–1644. DOI: 10.1136/heartjnl-2012-303237.
9. Ranthe MF, Diaz LJ, Behrens I, Bundgaard H, Simonsen J, Melbye M, Boyd HA. Association between pregnancy losses in women and risk of atherosclerotic disease in their relatives: a nationwide cohort study†. *Eur Heart J*. 2016;37:900–907. DOI: 10.1093/eurheartj/ehv549.
10. Colley E, Hamilton S, Smith P, Morgan NV, Coomarasamy A, Allen S. Potential genetic causes of miscarriage in euploid pregnancies: a systematic review. *Hum Reprod Update*. 2019;25:452–472. DOI: 10.1093/humupd/dmz015.
11. Kasak L, Punab M, Nagirnaja L, Grigorova M, Minajeva A, Lopes AM, Punab AM, Aston KI, Carvalho F, Laasik E, et al. Bi-allelic recessive loss-of-function variants in FANCM cause non-obstructive azoospermia. *Am J Hum Genet*. 2018;103:200–212. DOI: 10.1016/j.ajhg.2018.07.005.
12. Sulonen A-M, Ellonen P, Almusa H, Lepistö M, Eldfors S, Hannula S, Miettinen T, Tyynismaa H, Salo P, Heckman C, et al. Comparison of solution-based exome capture methods for next generation sequencing. *Genome Biol*. 2011;12:R94. DOI: 10.1186/gb-2011-12-9-r94.
13. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. *Bioinformatics*. 2014;30:2114–2120. DOI: 10.1093/bioinformatics/btu170.
14. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*. 2011;27:2987–2993. DOI: 10.1093/bioinformatics/btr509.
15. Ye K, Schulz MH, Long Q, Apweiler R, Ning Z. Pindel: a pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics*. 2009;25:2865–2871. DOI: 10.1093/bioinformatics/btp394.
16. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38:e164. DOI: 10.1093/nar/gkq603.
17. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581:434–443. DOI: 10.1038/s41586-020-2308-7.
18. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536:285–291. DOI: 10.1038/nature19057.
19. Wilfert AB, Chao KR, Kaushal M, Jain S, Zollner S, Adams DR, Conrad DF. Genome-wide significance testing of variation from single case exomes. *Nat Genet*. 2016;48:1455–1461. DOI: 10.1038/ng.3697.

20. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. Integrative genomics viewer. *Nat Biotechnol.* 2011;29:24–26. DOI: 10.1038/nbt.1754.

21. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405–424. DOI: 10.1038/gim.2015.30.

22. Kasak L, Rull K, Sober S, Laan M. Copy number variation profile in the placental and parental genomes of recurrent pregnancy loss families. *Sci Rep.* 2017;7:45327. DOI: 10.1038/srep45327.

23. Nagirnaja L, Palta P, Kasak L, Rull K, Christiansen OB, Nielsen HS, Steffensen R, Esko T, Remm M, Laan M. Structural genomic variation as risk factor for idiopathic recurrent miscarriage. *Hum Mutat.* 2014;35:972–982. DOI: 10.1002/humu.22589.

24. Robbins SM, Thimm MA, Valle D, Jelin AC. Genetic diagnosis in first or second trimester pregnancy loss using exome sequencing: a systematic review of human essential genes. *J Assist Reprod Genet.* 2019;36:1539–1548. DOI: 10.1007/s10815-019-01499-6.

25. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res.* 2019;47:D886–D894. DOI: 10.1093/nar/gky1016.

26. Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, Bottelli G, Cerrone M, Leonardi S. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. *JAMA.* 2005;294:2975–2980. DOI: 10.1001/jama.294.23.2975.

27. Schwartz PJ, Ackerman MJ, Antzelevitch C, Bezzina CR, Borggrefe M, Cuneo BF, Wilde AAM. Inherited cardiac arrhythmias. *Nat Rev Dis Primers.* 2020;6:58. DOI: 10.1038/s41572-020-0188-7.

28. Alders M, Bikker H, Christiaans I. Long QT syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, Amemiya A, eds. *Genereviews®* [Internet]. Seattle, WA: University of Washington, Seattle; 2018.

29. Priori SG, Barhanin J, Hauer RNW, Haverkamp W, Jongsma HJ, Kleber AG, McKenna WJ, Roden DM, Rudy Y, Schwartz K, et al. Genetic and molecular basis of cardiac arrhythmias: impact on clinical management parts I and II. *Circulation.* 1999;99:518–528. DOI: 10.1161/01.CIR.99.4.518.

30. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, Blom N, Brugada J, Chiang C-E, Huikuri H, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm.* 2013;10:1932–1963. DOI: 10.1016/j.hrthm.2013.05.014.

31. Crotti L, Tester DJ, White WM, Bartos DC, Insolia R, Besana A, Kunic JD, Will ML, Velasco EJ, Bair JJ, et al. Long QT syndrome-associated mutations in intrauterine fetal death. *JAMA.* 2013;309:1473–1482. DOI: 10.1001/jama.2013.3219.

32. Munroe PB, Addison S, Abrams DJ, Sebire NJ, Cartwright J, Donaldson I, Cohen MM, Mein C, Tinker A, Harmer SC, et al. Postmortem genetic testing for cardiac ion channelopathies in stillbirths. *Circ Genom Precis Med.* 2018;11:e001817. DOI: 10.1161/CIRCGEN.117.001817.

33. Sahlin E, Green A, Gustavsson P, Lieden A, Nordenskjold M, Papadogiannakis N, Pettersson K, Nilsson D, Jonasson J, Iwarsson E. Identification of putative pathogenic single nucleotide variants (SNVs) in genes associated with heart disease in 290 cases of stillbirth. *PLoS One.* 2019;14:e0210017. DOI: 10.1371/journal.pone.0210017.

34. Goldenberg I, Moss AJ, Zareba W. QT interval: how to measure it and what is "normal." *J Cardiovasc Electrophysiol.* 2006;17:333–336. DOI: 10.1111/j.1540-8167.2006.00408.x.

35. Sanguinetti MC, Jurkiewicz NK, Scott A, Siegl PK. Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes: mechanism of action. *Circ Res.* 1991;68:77–84. DOI: 10.1161/01.RES.68.1.77.

36. Han W, Wang Z, Nattel S. Slow delayed rectifier current and repolarization in canine cardiac Purkinje cells. *Am J Physiol Heart Circ Physiol.* 2001;280:H1075–H1080. DOI: 10.1152/ajpheart.2001.280.3.H1075.

37. Liin SI, Barro-Soria R, Larsson HP. The KCNQ1 channel—remarkable flexibility in gating allows for functional versatility. *J Physiol.* 2015;593:2605–2615. DOI: 10.1113/jphysiol.2014.287607.

38. Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current. *Nature.* 1996;384:78–80.

39. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature.* 1996;384:80–83. DOI: 10.1038/384080a0.

40. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet.* 1996;12:17–23. DOI: 10.1038/ng0196-17.

41. Laskowski RA, Stephenson JD, Sillitoe I, Orengo CA, Thornton JM. VarSite: disease variants and protein structure. *Protein Sci.* 2020;29:111–119. DOI: 10.1002/pro.3746.

42. Thompson E, Eldstrom J, Fedida D. Hormonal signaling actions on Kv7.1 (KCNQ1) channels. *Annu Rev Pharmacol Toxicol.* 2021;61:381–400. DOI: 10.1146/annurev-pharmtox-010919-023645.

43. Yang T, Kanki H, Roden DM. Phosphorylation of the IKs channel complex inhibits drug block: novel mechanism underlying variable antiarrhythmic drug actions. *Circulation.* 2003;108:132–134.

44. Cuneo BF, Kaizer AM, Clur SA, Swan H, Herberg U, Winbo A, Rydberg A, Haugaa K, Etheridge S, Ackerman MJ, et al. Mothers with long QT syndrome are at increased risk for fetal death: findings from a multicenter international study. *Am J Obstet Gynecol.* 2020;222:263.e1–263.e11. DOI: 10.1016/j.ajog.2019.09.004.

45. McCallum LA, Pierce SL, England SK, Greenwood IA, Tribe RM. The contribution of Kv7 channels to pregnant mouse and human myometrial contractility. *J Cell Mol Med.* 2011;15:577–586. DOI: 10.1111/j.1582-4934.2010.01021.x.

46. Mills TA, Greenwood SL, Devlin G, Shweikh Y, Robinson M, Cowley E, Hayward CE, Cottrell EC, Tropea T, Brereton MF, et al. Activation of Kv7 channels stimulates vasodilatation of human placental chorionic plate arteries. *Placenta.* 2015;36:638–644. DOI: 10.1016/j.placenta.2015.03.007.

47. Wilkins-Haug L. Genetic innovations and our understanding of stillbirth. *Hum Genet.* 2020;139:1161–1172. DOI: 10.1007/s00439-020-02146-2.

48. Marcondes L, Crawford J, Earle N, Smith W, Hayes I, Morrow P, Donoghue T, Graham A, Love D, Skinner JR, et al. Long QT molecular autopsy in sudden unexplained death in the young (1–40 years old): lessons learnt from an eight year experience in New Zealand. *PLoS One.* 2018;13:e0196078. DOI: 10.1371/journal.pone.0196078.

49. Luo Y, Kumar P, Mendelson CR. Estrogen-related receptor gamma (ERRgamma) regulates oxygen-dependent expression of voltage-gated potassium (K+) channels and tissue kallikrein during human trophoblast differentiation. *Mol Endocrinol.* 2013;27:940–952.

50. Itoh H, Berthet M, Fressart V, Denjoy I, Maugenre S, Klug D, Mizusawa Y, Makiyama T, Hofman N, Stallmeyer B, et al. Asymmetry of parental origin in long QT syndrome: preferential maternal transmission of KCNQ1 variants linked to channel dysfunction. *Eur J Hum Genet.* 2016;24:1160–1166. DOI: 10.1038/ejhg.2015.257.

51. American Cancer Society. *Cancer Facts & Figures 2020.* American Cancer Society; 2020.

52. Bagnes C, Panchuk PN, Recondo G. Antineoplastic chemotherapy induced QTc prolongation. *Curr Drug Saf.* 2010;5:93–96.

53. Diemberger I, Massaro G, Cubelli M, Rubino D, Quercia S, Martignani C, Ziacchi M, Biffi M, Bernardi A, Cacciari N, et al. Repolarization effects of multiple-cycle chemotherapy and predictors of QTc prolongation: a prospective female cohort study on >2000 ECGs. *Eur J Clin Pharmacol.* 2015;71:1001–1009.

54. Lin Y, Yu H, Liu F, Chen C, Zhang Y, Wang B, Yang Y, Liu Y, Zhang L, Xia Y. Hospitalized cancer patients with acquired long QT syndrome-a matched case-control study. *Cardiooncology.* 2020;6:3. DOI: 10.1186/s40959-020-0057-2.

55. Barsheshet A, Goldenberg I, O-Uchi J, Moss AJ, Jons C, Shimizu W, Wilde AA, McNitt S, Peterson DR, Zareba W, et al. Mutations in cytoplasmic loops of the KCNQ1 channel and the risk of life-threatening events: implications for mutation-specific response to beta-blocker therapy in type 1 long-QT syndrome. *Circulation.* 2012;125:1988–1996. DOI: 10.1161/CIRCULATIONAHA.111.048041.

56. Dixit G, Dabney-Smith C, Lorigan GA. The membrane protein KCNQ1 potassium ion channel: functional diversity and current structural insights. *Biochim Biophys Acta Biomembr.* 2020;1862:183148. DOI: 10.1016/j.bbamem.2019.183148.

57. Lundquist AL, Turner CL, Ballester LY, George AL Jr. Expression and transcriptional control of human KCNE genes. *Genomics*. 2006;87:119–128. DOI: 10.1016/j.ygeno.2005.09.004.

58. Odening KE, Koren G. How do sex hormones modify arrhythmogenesis in long QT syndrome? Sex hormone effects on arrhythmogenic substrate and triggered activity. *Heart Rhythm*. 2014;11:2107–2115. DOI: 10.1016/j.hrthm.2014.06.023.

59. Christine Knickmeyer R, Baron-Cohen S. Fetal testosterone and sex differences. *Early Hum Dev*. 2006;82:755–760. DOI: 10.1016/j.earlhumdev.2006.09.014.

60. Smith GC, Wood AM, Pell JP, Hattie J. Recurrent miscarriage is associated with a family history of ischaemic heart disease: a retrospective cohort study. *BJOG*. 2011;118:557–563. DOI: 10.1111/j.1471-0528.2010.02890.x.

61. Kessous R, Shoham-Vardi I, Pariente G, Sergienko R, Holcberg G, Sheiner E. Recurrent pregnancy loss: a risk factor for long-term maternal atherosclerotic morbidity? *Am J Obstet Gynecol*. 2014;211:414.e1-414.e11. DOI: 10.1016/j.ajog.2014.05.050.

62. Schnell F, Behar N, Carre F. Long-QT syndrome and competitive sports. *Arrhythm Electrophysiol Rev*. 2018;7:187–192. DOI: 10.15420/aer.2018.39.3.

63. Woosley RL, Black K, Heise CW, Romero K. Crediblemeds.Org: what does it offer? *Trends Cardiovasc Med*. 2018;28:94–99.

64. Fazio G, Vernuccio F, Grutta G, Re GL. Drugs to be avoided in patients with long QT syndrome: focus on the anaesthesiological management. *World J Cardiol*. 2013;5:87–93. DOI: 10.4330/wjc.v5.i4.87.

65. Maffucci P, Bigio B, Rapaport F, Cobat A, Borghesi A, Lopez M, Patin E, Bolze A, Shang L, Bendavid M, et al. Blacklisting variants common in private cohorts but not in public databases optimizes human exome analysis. *Proc Natl Acad Sci USA*. 2019;116:950–959. DOI: 10.1073/pnas.1808403116.

SUPPLEMENTAL MATERIAL

Data S1.

Clinical Phenotyping

Proband

Clinical assessment of the proband was performed at the Women's Clinic of Tartu University Hospital (TUH, Table S1). Laboratory test were performed at the United Laboratories, TUH.

Table S1. Maternal clinical characteristics

	Maternal age at assessment (years)	Results	Reference
Lifestyle factors			
Body mass index (non-pregnant state)	25-40	22-24 kg/m^2	19-25 kg/m^2
Smoking		never	
Diet		no restrictions	
Anatomical factors			
Ultrasonography	26-40, every 1-2 year	normal	
Hysteroscopy	26	normal	
Hormonal factors			
Thyroid stimulating hormone	26; 38	1.80; 2.13 mU/L	0.27 ... 4.20 mU/L
Free thyroxine	26; 38	17.5; 14.6 pmol/L	12.0 ... 22.0 pmol/L
Prolactin	26; 38	334; 256 mU/L	102–496 mU/L
Progesterone (luteal phase)	26; 33; 38	34.5, 30.9; 41.6 nmol/L	5.30 ... 86.00 nmol/L
Autoantibodies			
Anticardiolipin antibodies	26	4 IU/mL	<12 IU/mL
Anticardiolipin antibodies IgM	38	0.9 kU/L	<10 kU/L
Anticardiolipin antibodies IgM	38	1.1 kU/L	<10 kU/L
Beta-2 glucoprotein antibodies	33	negative	negative
Beta-2 glucoprotein antibodies IgM	38	<2.9 kU/L	<10 kU/L
Beta-2 glucoprotein antibodies IgG	38	0.7 kU/L	<10 kU/L
TPO- IgG	26, 38	17; 19 kU/L	<34 kU/L
Lupus antibodies	38	negative	negative
Blood coagulation			
Protein S antigen, free	26	94	62-146%
Protein C activity	26	133	75-135%
Antithrombin III activity	26	125	75-135%
Other tests			
Homocysteine	26;38	7.5; 9.3 µmol/L	<12.0 µmol/L

Fasting glucose	every pregnancy	< 5.1 mmol/l	<5.1 mmol/L
Vitamin D	26; 38	75; 81 nmol/L	>50 nmol/L
Urogenital tract infections			
Chlamydiosis	every pregnancy	negative	negative
Gonorrhoa	every pregnancy	negative	negative
Trichomonosis	26;38	negative	negative
Mycoplasmosis	26; 38	negative	negative
HIV	every pregnancy	negative	negative
Genetic factors			
Karyotype of both partners	26/32 partner	46 XX/46 XY	46 XX/46 XY
Factor V (Leiden) p.Arg506Gln, rs60254	26	Major allele	
Factor II (prothrombin) c.G20210A, rs179996341	26	Major allele	

Electrocardiograms

ECG report of the proband was initially assessed by Dr. Anne Kirss, a specialist in internal diseases and obstetric medicine at the Women's Clinic of Tartu University Hospital.

Additionally, the ECG reports were re-analyzed using Medilog Darwin Enterprise software ver. 2.9.2 (Schiller AG, Switzerland) and evaluated by Dr. Piibe Muda, a cardiologist specialized to rhythmology (Dept. of Clinical Physiology, Cardiology Clinic of Tartu University Hospital).

The proband has been subjected to ECG twice:
- at the age of 37 (heart rate 60 beats per minute, sinus rhythm, no abnormal re- and depolarization)
- at the age of 41 (see pp.3-6 detailed clinical reports, including Holter monitoring).

QTc was estimated using the Bazett formula.[64] For females, normal range of QTcB is considered <450 ms, borderline 451-470 and prolonged >470 ms. For children under the age of 15, normal range of QTcB is considered <440 ms, borderline 441-460 and prolonged >460 ms.

Gender	Female	Device ID	TUKNK1	
Ethnicity	Undefined	Acq. tech.		
Height		Acq. dept.		
Weight		Acq. inst.		

Medication
Remark

HR	84 bpm				
		RR	713 ms	Sinus rhythm	
		P	101 ms	Left atrial abnormality	
		PR	173 ms	Normal electrical axis	
P axis	62°	QRS	72 ms	T abnormality (inversion)	
QRS axis	64°	QT	401 ms	T abnormality in septal leads	
T axis	52°	QTcB	475 ms	Prolonged QT	
				Abnormal ECG	

Unconfirmed report

Abnormal

I
II
III
aVR
aVL
aVF
V1
V2
V3
V4
V5
V6

25 mm/s, 5 mm/mV

LP 40Hz, AC 50Hz

24-hour Holter-monitoring:

SA TÜ Kliinikum
Südamekliinik, rütmihäirete os.
Puusepa 8, F507
Tartu 50406

PROBAND

Patient Details

Name		Case number	
ID	PROBAND	Rec. start	11.06.2020 09:47:58
Age		Length	24:00:15 valid: 23:54:54
Gender		Recorder	AR12plus BT (12584/6.6 / 3.20)
Address		Ref.Doc.	
Phone		Contact	

Reason f. rec	
Current Therapy	
Recom. Therapy	

General

Beats	108880
V beats	1 (0,00%) PROBAND
Normal beats	108879 (100,00%)
Paced beats	0 (0,00%)
BBB	0 (0,00%)
Junction	0 (0,00%)
AFib	0
AFL	0
Artefacts%	0,37%
Length:	
ECG 1	23:52:27
ECG 2	23:51:39
ECG 3	23:53:39

Heartrate and HRV

Min. HR / maxRR	52 bpm @ 07:23:23 / 1215,5 ms
Max. HR / minRR	137 bpm @ 15:30:24 / 400,7 ms
Min. SinHR /maxNN	51,9 bpm / 1215,5 ms
Ø HR	76 bpm
Ø HR Day	80 bpm
Ø HR Night	70 bpm
Beats in Tachy	506 (125 bpm) 0,3%
Beats in Brady	0 (45 bpm) 0%
Longest Pause	--- sec @ ---
Longest QTc	40,4 sec / QTc: 534 msec @ 12.06.2020 02:10

Ventricular Events

V ectopic beats	1
Couplets	Mono: 0 Poly: 0
Triplets	0
Isolated V	0
Bigemini	0
Trigemini	0
VT	0
Most severe VT	
NSVT	0
Salvo	0
IVR	0

Supraventricular Events

Pause	0
Tachycardia	3
Most severe SVT	130,8 sec (defined by length) with 137 bpm @ 11.06.2020 15:29
Bradycardia	0
Most severe Brady	--- sec
SV Couplet	0
SV Triplet	0
PSVT	0
Irreg. Rhythm	0
SVES	2 (0,00%)
N-SVES	108877 (100,00%)

Diagnosis

Normal sinus rhythm, no pauses, daily average 76 beats per minute. A few extrasystols. Borderline QT intervall. QT-c is prolonged at night, the longest QTc 534 ms (2:10 am). The longest QTc 508 ms at day time (incl. after daily exercises).

Details of Holter monitoring

Heart Rate Trend

max HR: **137bpm @ 15:30:24**
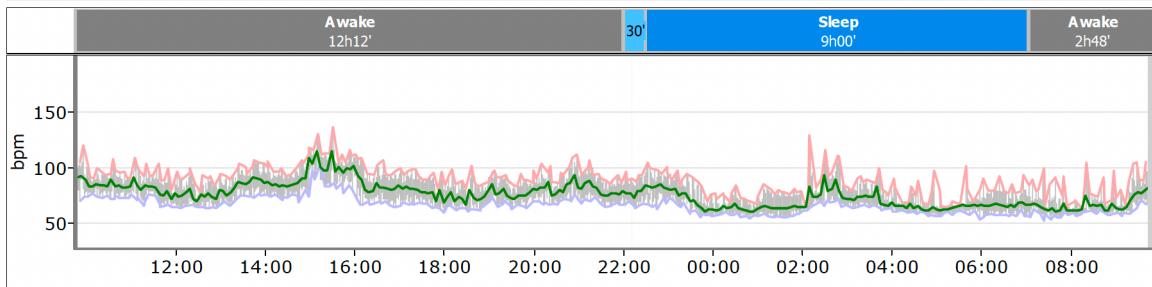
PR Trend
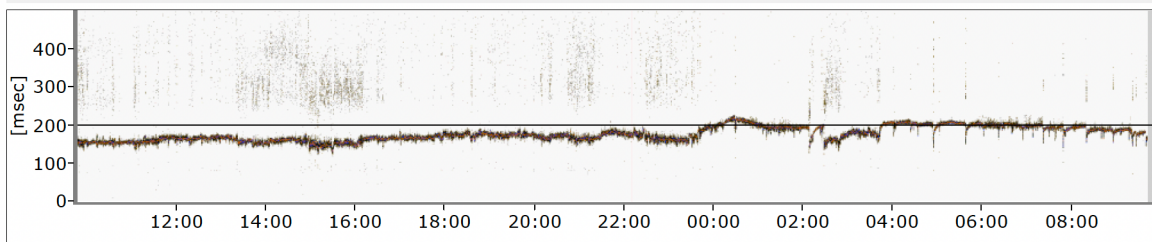
Arrhythmia Trend
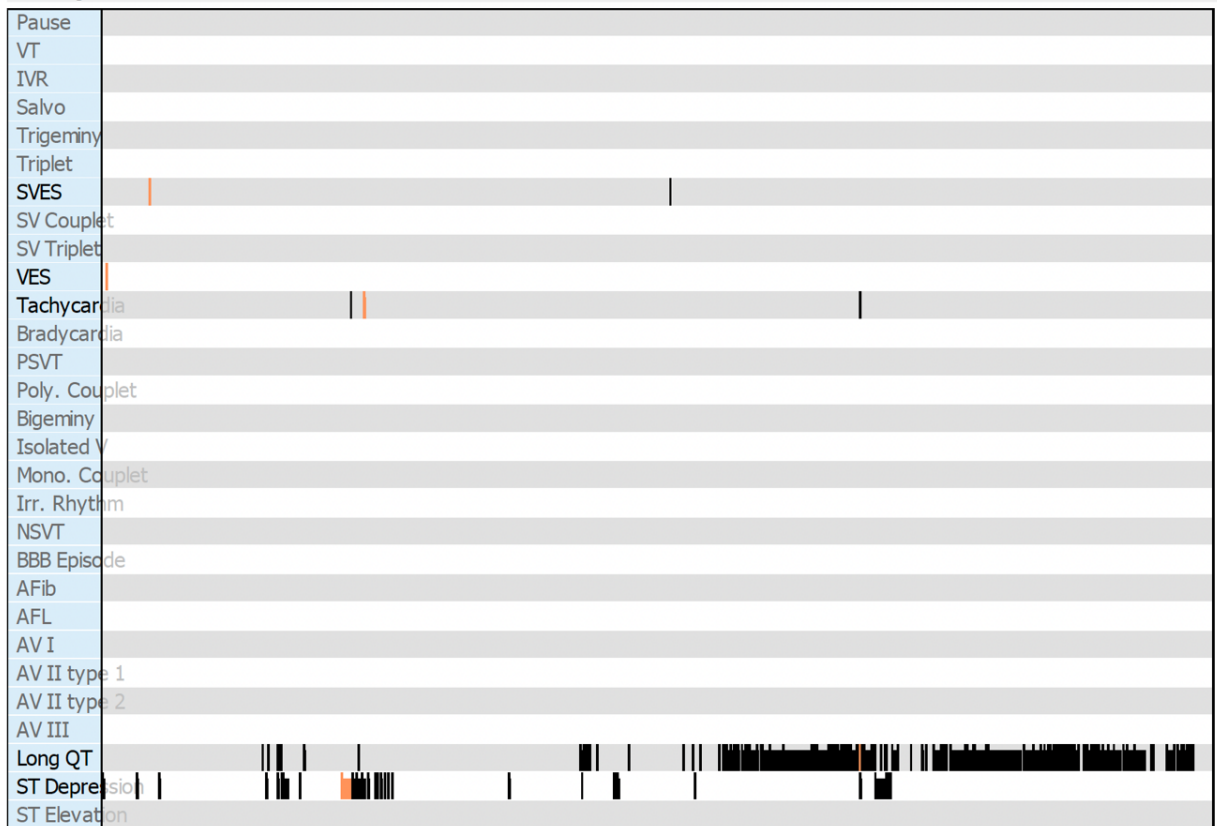
QT summary table

Time	Total beats	QTc min	QTc max	QTc mean
Entire rec.	92092	367	534	471
Day	56607	367	508	465
Night	35485	372	534	480
09:47:58	3829	397	508	461
10:47:58	4279	442	482	460
11:47:58	4332	421	480	464
12:47:58	4007	392	501	466
13:47:58	3870	387	499	452
14:47:58	727	384	492	431
15:47:58	2910	367	479	461
16:47:58	4405	427	470	455
17:47:58	4158	443	477	458
18:47:58	4377	451	486	466
19:47:58	4308	403	496	474
20:47:58	3950	369	491	460
21:47:58	4123	399	502	468
22:47:58	4192	398	506	474
23:47:58	3806	467	505	490
00:47:58	3795	472	506	488
01:47:58	3867	380	534	472
02:47:58	4115	372	497	474
03:47:58	3931	465	502	484
04:47:58	3791	402	506	488
05:47:58	3966	468	493	484
06:47:58	3894	439	498	483
07:47:58	3816	414	495	480
08:47:58	3644	440	492	478

Details of pregnancy loss pathology reports

All pathology examinations were performed at the Pathology Department of Tartu University Hospital and revised by Dr. Liis Salumäe.

Case III-5

Fetal death at 14 weeks and 5 days

Vanishing twin at 6-7 gestational weeks

Placenta: weight 20 g, focal delayed maturation and circulatory disorders, no inflammation, placental age corresponds to 13-14 gestational weeks

Fetus: weight 44 g, length 16.5 cm, female, no malformations

Case III-9

Fetal death at 17 gestational weeks

Placenta: weight 56 g, stroma of chorionic villi contain abundant Hoffbauer cells, focal thrombotic vasculopathy, abundant erythroblasts in placental vessels that may indicate fetal anemia, no inflammation.

Fetus: weight 56 g (corresponds to 15-16 weeks), length 15.4 cm (corresponds to 16-17 weeks), rump length 10.5 cm, foot 1.7 cm, female, no malformations. Lung tissue contains abundant erythroblasts, which may indicate fetal anemia.

Case III-13

Fetal death at 18 gestational weeks

Placenta and umbilical cord: weight 91.9 g, size 12x9.5x1.5 cm, marginal insertion of umbilical cord, length of umbilical cord 52 cm, coiling index 0.61 (normal range 0.07-0.3). Some typical postmortem changes: minimal villous fibrosis, no inflammation.

Fetus: weight 174 g (corresponds to 17-18 weeks), length 21.5 cm (corresponds to 18 weeks), rump length 15 cm, foot 2.6 cm, head circumference 14 cm, abdominal circumference 13 cm. Visual examination: left hand mild clinodactyly, low-lying ears.

Brain-liver ratio=4.7 (normal 3), indicates asymmetric fetal growth restriction. No malformations.

Detailed health data for live born children

Data about pregnancy course and deliveries were obtained from the medical case database of Tartu University Hospital and personal contact. Health data for children was obtained from interviews with the proband and electronic medical case history. Cardiac assessment of two children (III-11, III-12), both heterozygous carriers of the *KCNQ1* c.518T>A variant, was performed by Dr. Kristel Köbas, pediatric cardiologist, Children's Clinic of Tartu University Hospital.

III-1

Uncomplicated pregnancy and vaginal birth at 39 weeks, birth weight 3300 g, male.

According to maternal data, there was fetal hypoxia and meconial staining of amniotic fluid.

Atopic dermatitis and asthma since 1 months after birth, D-vitamin deficiency during infancy and minor scoliosis.

III-2

Uncomplicated pregnancy and vaginal birth at 38 weeks, birth weight 3600 g, male.

No health problems during infancy and childhood except sport injuries: ear injury (at the age of 11 years), fracture of left big toe (at the age of 16 years).

III-10

Uncomplicated pregnancy and vaginal birth at 37 weeks, birth weight 3364 g, male.

During pregnancy, low molecular-weight heparin enoxaparin was administered due to focal delayed maturation of villi and circulatory disorders for pregnancy loss case at 14 weeks and 5 days (III-5) and focal thrombotic vasculopathy for pregnancy loss at 17 weeks (III-9).

Atopic dermatitis during infancy, scarlet fever (at the age of 6), injury of elbow (at the age of 4), surgical repair of umbilical hernia (at the age of 8).

III-11

Uncomplicated pregnancy and vaginal birth at 40 weeks, birth weight 3395 g, male.

During pregnancy, low molecular-weight heparin enoxaparin was administered.

During infancy bronchitis and asthma, surgery due to appendicitis (at the age of 4 years), sport injuries.

Heterozygous carrier of *KCNQ1* c.518T>A variant.

Cardiac assessment at the age of 11:

No complaints, able to perform all physical activities and sports. No extra physical training.

Weight 35 kg, height 155 cm, blood pressure systolic 106 mmHg, diastolic 66 mmHg.

Lying position: mild sinus arrythmia, heart rate 64 beats per minute

Standing position: normal rhythm, heart rate 80 beats per minute

No pathological murmurs.

ECG lying: sinus rhythm heart rate 66 beats per minute, axis +79 degree, PR 120ms, QRS 82ms, slightly prolonged QTcB 442 ms. De- and repolarization normal.

ECG standing: sinus rhythm heart rate 71 beats per minute, axis +73 degree, PR 115 ms, QRS 72ms, normal QTcB 438 ms.

Echocardiography: Four normal size chambers, no septal defects. The myocardial thickness of left ventricle normal, fractional shortening 39% (normal), ejection fraction 70% (normal). Normal function of valves. Physiological regurgitation at tricuspid valve and pulmonal valve. Pulmonal pressure normal.

Conclusion: No pathology.

III-11 lying position

♥ 66 bpm

Intervals		Axis	
RR	909 ms	P axis	44 °
P	87 ms	QRS axis	79 °
PR	120 ms	T axis	58 °
QRS	82 ms		
QT	421 ms	**LVH Criteria**	
QTcB	442 ms	Sokolow-Lyon	5.11 mV
QTcF	435 ms	Cornell	1.26 mV
QTm	432 ms	Lewis	-1.50 mV
QTlc	435 ms	Romhilt-Estes	3

Created by Arst

Lying position: sinus rhythm, heart rate 66 beats per minute. Normal electrical axis 79 degrees, PR 120 ms, QRS 82 ms, slightly prolonged QTcB 442 ms. Repolarization normal, no hypertrophia.

III-11 standing position

71 bpm

Intervals

		Axis	
RR	843 ms	P axis	44 °
P	85 ms	QRS axis	73 °
PR	115 ms	T axis	54 °
QRS	72 ms		
QT	402 ms	**LVH Criteria**	
QTcB	438 ms	Sokolow-Lyon	5.24 mV
QTcF	426 ms	Cornell	1.38 mV
QTm	421 ms	Lewis	-1.03 mV
QTlc	426 ms	Romhilt-Estes	3

Created by Arst

Standing position: sinus rhythm, heart rate 71 beats per minute. Normal electrical axis 73 degrees, PR 115 ms, QRS 72 ms, normal QTcB 438 ms.

25 mm/s, 10 mm/mV

LP 40Hz, AC 50Hz

III-12

Uncomplicated pregnancy and vaginal birth at 38 weeks, birth weight 3344 g, female.

During pregnancy, low molecular-weight heparin enoxaparin was administered.

Napkin dermatitis in infancy.

Congenital ventricular septal defect „swiss cheese" type - multiple small muscular defects.

The defect was detected accidentally by auscultation at the age of 3 (2-degree murmur), spontaneous closure by age 7.

Heterozygous carrier of *KCNQ1* c.518T>A variant

Cardiac assessment at the age of 7:

No complaints, able to perform all physical activities.

Weight 19 kg, height 123 cm, blood pressure systolic 95 mmHg, diastolic 61 mmHg.

Mild systolic murmur and mild respiratory arrhythmia.

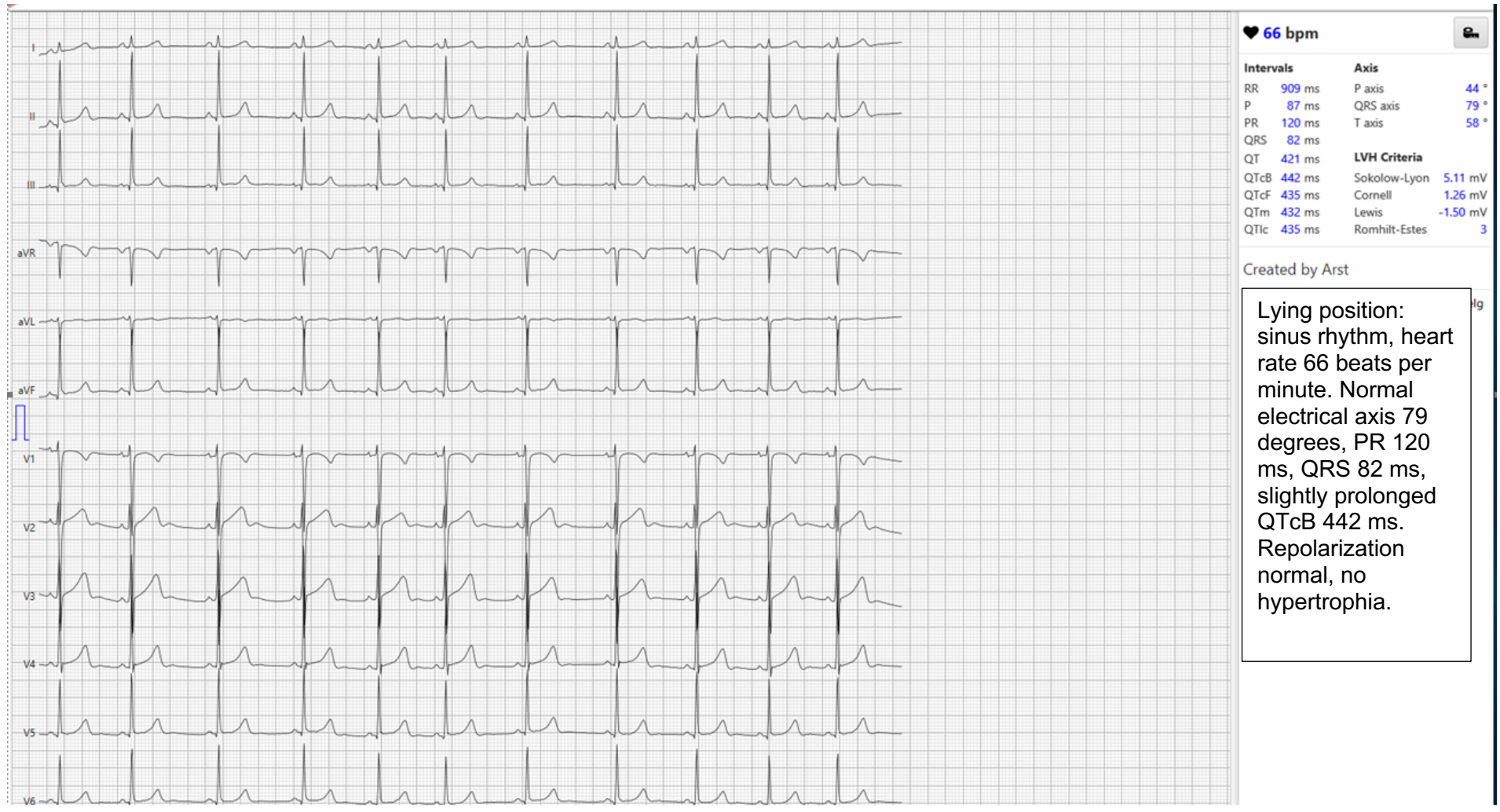ECG: sinus rhythm, heart rate 78 beats per minute, axis +46 degree. De- and repolarization normal.

Echocardiography: Four normal size chambers, no septal defects. The myocardial thickness of left ventricle normal, fractional shortening 37% (normal), ejection fraction 67% (normal). Normal function of valves. Physiological regurgitation at tricuspid valve and pulmonal valve. Pulmonal pressure normal. Extra chordae tendineae were detected at left ventricle that may explain mild murmur.

Conclusion: No pathology

Cardiac assessment at the age of 8:

Occasional chest pain at resting state, occurring more frequently during school period. She is able to perform all physical activities without any complaints.

Weight 24 kg, height 133 cm, blood pressure systolic 101 mmHg, diastolic 66 mmHg.

No pathological murmurs at auscultation.

ECG: sinus rhythm, heart rate 68 beats per minute, PE 133 ms, QRS 69 ms, QTcB 427 ms, axis +57 degree. De- and repolarization normal.

Echocardiography: Four normal size chambers, no septal defects. The myocardial thickness of left ventricle normal. Normal function of valves. Physiological regurgitation at mitral valve. Pulmonal pressure normal.

Conclusion: No pathology

24-hour Holter: Normal sinus rhythm. 24-h average heart rate 76 beats per minute, minimal 47 beats per minute during sleep, maximum 153 beats per minute during exercise.

III-12
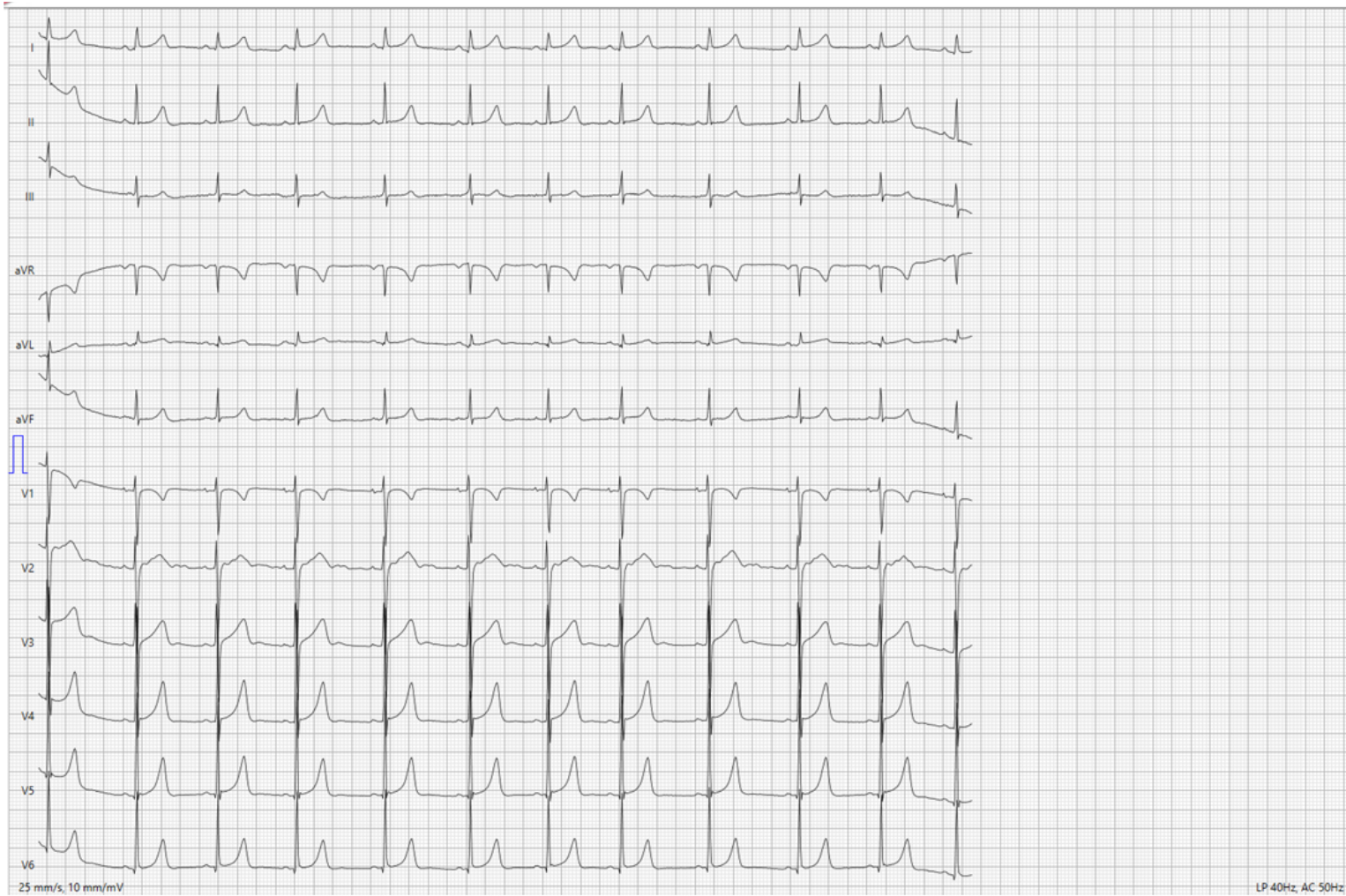
♥ 68 bpm

Intervals

		Axis	
RR	888 ms	P axis	22 °
P	89 ms	QRS axis	57 °
PR	133 ms	T axis	30 °
QRS	69 ms		
QT	402 ms	**LVH Criteria**	
QTcB	427 ms	Sokolow-Lyon	5.26 mV
QTcF	418 ms	Cornell	1.95 mV
QTm	416 ms	Lewis	0.18 mV
QTlc	419 ms	Romhilt-Estes	--

Created by ETM V2.4.0.0

Sinus rhythm, heart rate 68 beats per minute. Normal electrical axis 57 degrees, PR 133 ms, QRS 69 ms, normal QTcB 427 ms. De- and repolarization normal.

25 mm/s, 10 mm/mV

LP 40Hz, AC 50Hz

Gathered health data of the proband's sister, who is living abroad (II-3)

The proband's sister (age 38) has experienced one empty sac pregnancy loss followed by two uncomplicated pregnancies and vaginal deliveries (at age 32 and 34). She has had appendectomy at the age of 10 and tonsillectomy at the age of 23. She was diagnosed with myocarditis (age 24) and thyroid gland cyst (27). During recent years, she has had complaints for occasional palpitations and faintness; however, no pathology was found at electrocardiogram (age 38, detailed report unavailable due to residency abroad). She was also diagnosed with magnesium deficiency (detailed data is not available) and oral supplementation of both magnesium and potassium improved her status.

Table S2. Final list of shared variants in all pregnancy loss samples (all in heterozygous state)

Chr	Start (hg19)	End	Ref	Alt	Rs number	Gene.refGene	ExonicFunc. refGene	gnomAD_ genomes_ALL	ExAC_ALL	1000g 2015aug_all	ACMG
1	77515945	77515945	A	G	rs779329661	*ST6GALNAC5*	missense	6.5E-05	8.2E-06	NA	VUS
1	170952626	170952626	T	C	rs201549824	*MROH9*	missense	7.0E-04	4.0E-04	NA	VUS
1	183876214	183876214	G	A	rs752600877	*RGL1*	missense	6.5E-05	2.0E-05	NA	VUS
1	201181256	201181256	C	G	NA	*IGFN1*	missense	NA	NA	NA	VUS
2	65543962	65543962	G	A	rs141237945	*SPRED2*	missense	7.0E-04	7.8E-05	2.0E-04	VUS
2	85012801	85012801	C	T	rs531681722	*DNAH6*	missense	1.0E-04	NA	NA	VUS
3	48667506	48667506	G	A	rs201569807	*SLC26A6*	missense	9.0E-04	5.0E-04	NA	VUS
3	151161276	151161276	C	T	rs770724810	*IGSF10*	missense	NA	1.6E-05	NA	VUS
5	14488046	14488046	G	A	rs773401747	*TRIO*	missense	6.0E-04	2.0E-04	NA	LB
5	36049244	36049244	A	G	rs200492917	*UGT3A2*	missense	2.0E-04	2.0E-04	NA	VUS
6	41621112	41621112	C	A	rs371572169	*MDFI*	stopgain	NA	6.6E-05	NA	VUS
6	43307372	43307380	GGTGGTGGA	-	rs563671976	*ZNF318*	nonframeshift deletion	2.0E-04	3.0E-04	6.0E-04	VUS
6	109752445	109752445	A	G	rs199983515	*PPIL6*	missense	3.0E-04	8.0E-04	6.0E-04	VUS
7	142749654	142749654	A	G	rs373694316	*OR6V1*	missense	9.7E-05	5.8E-05	NA	VUS
9	100693345	100693345	G	C	rs139664531	*HEMGN*	missense	7.0E-04	6.0E-04	NA	LB
11	102668000	102668000	G	A	rs773149802	*MMP1*	missense	NA	8.2E-06	NA	VUS
12	6939652	6939652	C	A	NA	*P3H3*	missense	6.5E-05	NA	NA	VUS
12	52828048	52828048	C	T	rs146288298	*KRT75*	missense	4.0E-04	4.0E-04	2.0E-04	VUS
13	20220901	20220901	G	C	NA	*MPHOSPH8*	missense	NA	NA	NA	VUS
13	78143586	78143586	G	C	rs1280854840	*SCEL*	missense	1.0E-04	NA	NA	VUS
14	71199844	71199844	G	A	rs149935990	*MAP3K9*	missense	1.0E-04	1.0E-04	NA	VUS
14	95053962	95053962	A	C	rs1274092862	*SERPINA5*	missense	5.0E-04	NA	NA	VUS
16	136766	136766	C	T	rs188724206	*NPRL3*	missense	6.5E-05	1.0E-04	NA	VUS
16	10637443	10637443	G	C	rs146745761	*EMP2*	missense	2.0E-04	9.1E-05	NA	VUS

16	67866375	67866375	G	A	rs892367026	*CENPT*	missense	NA	NA	NA	VUS
19	14518820	14518820	G	A	rs201291607	*ADGRE5*	missense	3.2E-05	2.5E-05	2.0E-04	VUS
19	58370042	58370042	C	T	NA	*ZNF587*	missense	NA	NA	NA	VUS
20	18286410	18286410	G	A	NA	*ZNF133*	missense	NA	NA	NA	VUS
20	36841679	36841679	G	A	rs867311806	*KIAA1755*	missense	3.2E-05	NA	NA	VUS
22	50298065	50298065	G	A	rs748537273	*ALG12*	missense	1.0E-04	1.0E-04	NA	VUS
22	50873472	50873472	G	A	rs144614869	*PPP6R2*	missense	4.0E-04	3.0E-04	NA	VUS
X	48752372	48752372	G	A	rs142117152	*TIMM17B*	missense	8.0E-04	1.0E-03	NA	VUS
X	68725840	68725840	G	A	rs201772031	*FAM155B*	missense	9.0E-04	4.0E-04	NA	VUS

LB, likely benign; VUS, variant of uncertain significance

Table S3. Top variants called with high confidence in the proband (II-1) by the PSAP pipeline (all in heterozygous state)

Chr	Start (hg19)	Ref	Alt	Rs number	Gene. wgEncode Gencode BasicV19	ExonicFunc. wgEncode Gencode BasicV19	gnomAD_ exome_ALL	gnomAD_ exome_NFE	ExAC_ALL	1000g 2015aug_ all	CADD13_ PHRED	PSAP score	ACMG
1	11718846	T	C	rs1259964802	FBXO44	missense	NA	NA	NA	NA	26	0.00351	VUS
1	156767115	C	G	NA	PRCC	missense	NA	NA	NA	NA	26	0.00087	VUS
2	74699557	T	A	rs751976686	MRPL53	missense	8.15E-06	0	NA	NA	24	0.00074	VUS
2	113147677	G	A	rs572623209	RGPD8	stopgain	6.00E-04	0.0003	5.00E-04	2.00E-04	35	0.00147	VUS
2	241570199	A	G	rs201512343	GPR35	missense	4.06E-06	8.96E-06	NA	NA	26	0.00450	VUS
3	48667506	G	A	rs201569807	SLC26A6	missense	7.00E-04	0.0007	5.00E-04	NA	28	0.00349	VUS
3	57456183	G	A	rs765440022	DNAH12	stopgain	1.00E-04	0.0002	9.25E-05	NA	39	0.00401	VUS
3	159943576	T	C	rs774658584	C3orf80	missense	7.00E-04	9.77E-05	1.00E-04	NA	27	0.00140	VUS
5	36049244	A	G	rs200492917	UGT3A2	missense	2.00E-04	0.0003	2.00E-04	NA	28	0.00119	VUS
6	41621112	C	A	rs371572169	MDFI	stopgain	5.32E-05	2.70E-05	6.61E-05	NA	38	0.00009	VUS
7	43594284	C	T	rs202007423	HECW1	missense	0.001	0.0001	6.00E-04	NA	33	0.00464	VUS
8	66619295	C	A	rs1316653370	MTFR1	missense	0	0	NA	NA	25	0.00297	VUS
11	2591898	T	A	rs199472695	KCNQ1	missense	NA	NA	NA	NA	29	0.00330	**LP***
11	60609622	C	T	rs199809991	CCDC86	stopgain	2.00E-04	0.0004	3.00E-04	NA	36	0.00144	VUS
11	118978769	G	C	rs772121984	DPAGT1	missense	4.54E-05	1.05E-05	2.67E-05	NA	28	0.00448	VUS
12	65237219	A	G	NA	TBC1D30	missense	NA	NA	NA	NA	24	0.00224	VUS
12	91449778	G	C	NA	KERA	missense	NA	NA	NA	NA	26	0.00072	VUS
13	96329519	C	T	rs746295509	DNAJC3	missense	2.13E-05	4.66E-05	2.56E-05	NA	23	0.00420	VUS
14	35182602	C	T	rs200536303	CFL2	missense	2.00E-04	0.0002	2.00E-04	NA	27	0.00048	VUS
14	75330314	A	G	rs199570102	PROX2	missense	8.00E-04	0.0005	9.00E-04	3.99E-04	24	0.00484	VUS
16	11781747	G	A	rs559217484	TXNDC11	missense	2.85E-05	2.69E-05	3.37E-05	2.00E-04	34	0.00180	VUS
16	15045631	G	A	rs201805072	NPIPA1	missense	3.00E-04	0.0001	2.00E-04	NA	20	0.00343	LB
16	68010634	C	T	rs199567405	DPEP3	missense	2.00E-04	5.38E-05	3.00E-04	2.00E-04	35	0.00027	VUS
16	89857848	C	T	rs371716550	FANCA	missense	4.07E-06	8.98E-06	8.46E-06	NA	32	0.00158	VUS
17	58143728	G	A	rs150364683	HEATR6	missense	6.00E-04	0.0006	5.00E-04	NA	35	0.00456	VUS
19	14518820	G	A	rs201291607	DDX39A	missense	2.44E-05	2.69E-05	2.49E-05	2.00E-04	22	0.00400	VUS

19	18392033	G	C	rs1293425649	*JUND*	missense	NA	NA	NA	NA	24	0.00146	VUS
19	55597880	G	T	rs200225675	*EPS8L1*	stopgain	2.00E-04	0.0005	4.00E-04	NA	42	0.00105	VUS
21	33739048	G	A	rs187485893	*URB1*	missense	5.00E-04	0.0006	6.00E-04	2.00E-04	34	0.00455	VUS
22	29927873	G	A	rs199572850	*THOC5*	missense	8.00E-04	0.0001	7.00E-04	3.99E-04	34	0.00079	VUS
X	117861610	G	A	NA	*IL13RA1*	stopgain	NA	NA	NA	NA	35	0.00004	VUS

*ACMG tags: PM1, PM2, PP2, PP3

LB, likely benign; LP, likely pathogenic; VUS, variant of uncertain significance

Table S4. Merged output of the top variants called with high confidence in the pregnancy loss samples (III-5, III-9, III-13) by the PSAP pipeline (all in heterozygous state)

Chr	Start (hg19)	Ref	Alt	Gene. wgEncode Gencode BasicV19	Rs number	ExonicFunc. wgEncode Gencode BasicV19	gnomAD_ exome_ALL	gnomAD_ exome_NFE	ExAC_ALL	1000g 2015aug_ all	CADD13_ PHRED	PSAP	ACMG	Number of carrier pregnancy losses
1	11718846	T	C	*FBXO44*	rs1259964802	missense	NA	NA	NA	NA	26	0.00351	VUS	2
1	16111074	C	A	*FBLIM1*	rs772470861	missense	4.08E-06	9.04E-06	8.24E-06	NA	34	0.00033	VUS	1
1	101005357	C	T	*GPR88*	rs1255606770	missense	8.54E-06	0	NA	NA	24	0.00070	VUS	1
1	113253469	C	T	*PPM1J*	rs146793493	missense	6.91E-05	8.96E-05	7.42E-05	NA	33	0.00253	VUS	1
1	120502048	G	A	*NOTCH2*	NA	missense	8.14E-06	0	NA	NA	29	0.00474	VUS	2
1	150621165	C	T	*GOLPH3L*	rs186291546	missense	5.00E-04	0.0008	4.00E-04	NA	34	0.00196	VUS	1
1	156767115	C	G	*PRCC*	NA	missense	NA	NA	NA	NA	26	0.00087	VUS	2
2	74699557	T	A	*MRPL53*	rs751976686	missense	8.15E-06	0	NA	NA	24	0.00074	VUS	2
2	113147677	G	A	*RGPD8*	rs572623209	stopgain	6.00E-04	0.0003	5.00E-04	2.00E-04	35	0.00147	VUS	1
2	219825198	C	T	*CDK5R2*	rs1447466372	missense	NA	NA	NA	NA	33	0.00017	VUS	2
2	228560627	T	G	*SLC19A3*	rs1473191700	missense	4.88E-05	0	NA	NA	27	0.00219	VUS	1
2	241570199	A	G	*GPR35*	rs201512343	missense	4.06E-06	8.96E-06	NA	NA	26	0.00450	VUS	2
3	46621330	G	C	*TDGF1*	rs148619685	missense	6.00E-04	0.0009	6.00E-04	2.00E-04	22	0.00345	LB	1
3	48667506	G	A	*SLC26A6*	rs201569807	missense	7.00E-04	0.0007	5.00E-04	NA	28	0.00349	VUS	3
3	130743495	G	A	*ASTE1*	rs140373702	missense	5.00E-04	0.0009	7.00E-04	NA	27	0.00334	VUS	2
3	159943576	T	C	*C3orf80*	rs774658584	missense	7.00E-04	9.77E-05	1.00E-04	NA	27	0.00140	VUS	3
4	76551105	T	G	*CDKL2*	NA	missense	NA	NA	NA	NA	26	0.00179	VUS	2
5	34824200	AAGC	-	*RAI14*	NA	frameshift deletion	NA	NA	NA	NA	33	0.00155	VUS	2
5	36049244	A	G	*UGT3A2*	rs200492917	missense	2.00E-04	0.0003	2.00E-04	NA	28	0.00119	VUS	3
5	39376818	C	T	*DAB2*	rs760327528	missense	1.63E-05	3.59E-05	2.48E-05	NA	33	0.00431	VUS	2
6	31683151	G	T	*LY6G6D*	rs536760002	missense	4.69E-05	9.51E-05	8.17E-05	2.00E-04	25	0.00050	VUS	2
6	41621112	C	A	*MDFI*	rs371572169	stopgain	5.32E-05	2.70E-05	6.61E-05	NA	38	0.00009	VUS	3
6	42179538	G	T	*MRPS10*	rs768228186	missense	4.07E-06	0	8.24E-06	NA	34	0.00046	VUS	1

7	43594284	C	T	*HECW1*	rs202007423	missense	0.001	0.0001	6.00E-04	NA	33	0.00464	VUS	2
7	107423534	A	G	*SLC26A3*	rs1471734047	missense	NA	NA	NA	NA	32	0.00116	VUS	2
8	68184086	C	T	*ARFGEF1*	rs1350123170	missense	NA	NA	NA	NA	27	0.00427	VUS	2
10	134941871	A	G	*GPR123*	rs145500903	missense	2.00E-04	0.0003	3.00E-04	NA	24	0.00491	VUS	1
11	2591898	T	A	*KCNQ1*	rs199472695	missense	NA	NA	NA	NA	29	0.00330	**LP**	2
11	60609622	C	T	*CCDC86*	rs199809991	stopgain	2.00E-04	0.0004	3.00E-04	NA	36	0.00144	VUS	2
11	64940060	G	A	*SPDYC*	rs147348885	missense	2.00E-04	0.0002	1.00E-04	NA	28	0.00086	VUS	1
11	118978769	G	C	*DPAGT1*	rs772121984	missense	4.54E-05	1.05E-05	2.67E-05	NA	28	0.00448	VUS	2
11	119243689	G	A	*USP2*	rs1344550169	missense	1.22E-05	0	NA	NA	24	0.00454	VUS	1
12	91449778	G	C	*KERA*	NA	missense	NA	NA	NA	NA	26	0.00072	VUS	2
12	109719496	G	A	*FOXN4*	rs759819045	missense	2.56E-05	2.87E-05	3.07E-05	NA	23	0.00094	VUS	1
13	96329519	C	T	*DNAJC3*	rs746295509	missense	2.13E-05	4.66E-05	2.56E-05	NA	23	0.00420	VUS	1
14	75277083	C	T	*YLPM1*	rs750118817	missense	4.07E-06	0	8.30E-06	NA	35	0.00280	VUS	1
16	136766	C	T	*MPG*	rs188724206	missense	7.41E-05	0.0001	1.00E-04	NA	34	0.00190	VUS	3
16	136766	C	T	*NPRL3*	rs188724206	missense	7.41E-05	0.0001	1.00E-04	NA	34	0.00209	VUS	3
16	11781747	G	A	*TXNDC11*	rs559217484	missense	2.85E-05	2.69E-05	3.37E-05	2.00E-04	34	0.00180	VUS	1
16	68010634	C	T	*DPEP3*	rs199567405	missense	2.00E-04	5.38E-05	3.00E-04	2.00E-04	35	0.00027	VUS	2
17	34105997	C	T	*MMP28*	rs199792911	missense	1.00E-04	0.0002	1.00E-04	NA	35	0.00079	VUS	1
17	38349661	G	A	*RAPGEFL1*	rs201057482	missense	2.03E-05	1.79E-05	1.65E-05	2.00E-04	21	0.00368	VUS	2
17	58143728	G	A	*HEATR6*	rs150364683	missense	6.00E-04	0.0006	5.00E-04	NA	35	0.00456	VUS	1
17	74681232	A	G	*MXRA7*	rs200298746	missense	1.00E-04	0.0002	2.00E-04	NA	26	0.00355	VUS	2
18	56016778	C	T	*NEDD4L*	rs1020866073	missense	NA	NA	NA	NA	35	0.00076	VUS	2
19	1881370	A	T	*ABHD17A*	rs201914248	missense	3.00E-04	0.0002	2.00E-04	NA	23	0.00103	VUS	1
19	2272808	G	T	*OAZ1*	rs534549628	missense	7.00E-04	5.08E-05	2.00E-04	2.00E-04	35	0.00023	VUS	1
19	8577958	C	T	*ZNF414*	rs749498595	missense	1.00E-04	0.0002	1.00E-04	NA	26	0.00216	VUS	2
19	14518820	G	A	*DDX39A*	rs201291607	missense	2.44E-05	2.69E-05	2.49E-05	2.00E-04	22	0.00400	VUS	3
19	18392033	G	C	*JUND*	rs1293425649	missense	NA	NA	NA	NA	24	0.00146	VUS	1
19	42355696	C	T	*DMRTC2*	rs141103699	stopgain	1.00E-04	0.0002	7.42E-05	NA	37	0.00041	VUS	2

19	55597880	G	T	*EPS8L1*	rs200225675	stopgain	2.00E-04	0.0005	4.00E-04	NA	42	0.00105	VUS	1
21	30959710	C	T	*GRIK1*	rs753443587	missense	4.07E-06	8.97E-06	8.27E-06	NA	29	0.00265	VUS	2
21	42807881	G	A	*MX1*	rs150271063	missense	4.00E-04	0.0008	4.00E-04	NA	31	0.00344	VUS	2
22	29927873	G	A	*THOC5*	rs199572850	missense	8.00E-04	0.0001	7.00E-04	3.99E-04	34	0.00079	VUS	2
X	48752372	G	A	*TIMM17B*	rs142117152	missense	6.00E-04	0.0009	0.001	NA	27	0.00224	VUS	3

LB, likely benign; LP, likely pathogenic; VUS, variant of uncertain significance
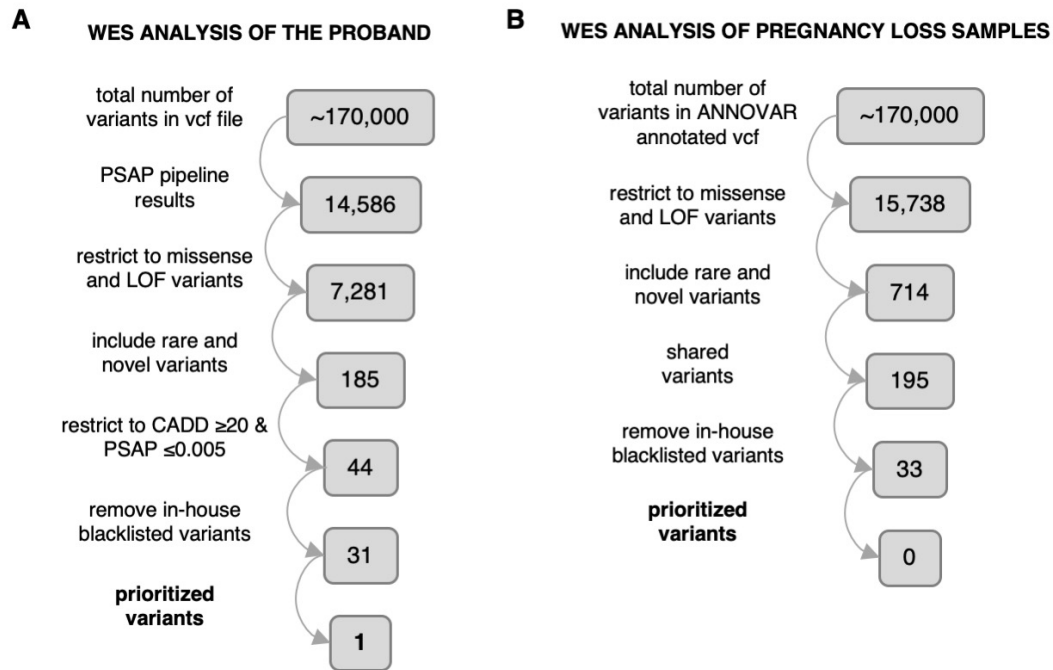
Figure S1. Overview of variant prioritizing of WES data in the proband by PSAP analysis and in the pregnancy loss samples by filtering all variants. VCF files contained approximately 170,000 variants.

(A) An updated version of the population sampling probability (PSAP) pipeline[19] was applied in order to prioritize potential causative variants from the WES data of the proband. A new feature in this implementation of PSAP is ethnicity-specific models. PSAP analysis pipeline resulted in 14,586 variants. The variants were prioritized to satisfy the following criteria: (i) missense and LoF variants, (ii) low minor allele frequency (MAF ≤0.001 ExAC, gnomAD and 1000GP) or a previously undescribed variant; (iii) the PSAP statistical significance value ≤0.005; the CADD score ≥20.

(B) VCF files of the pregnancy loss samples were annotated using ANNOVAR (version 20191024)[16]. Among all coding variants (exonic and splicing) synonymous and common variants with MAF of more than 0.1% in gnomAD[17] and ExAC[18] were removed. Only shared variants in all pregnancy loss samples were retained.

Next, for both parts of the analysis (A-B), in-house blacklisted variants were removed, i.e., variants corresponding to false signals generated by incomplete reference genome assembly, location in low-complexity regions, bioinformatic misprocessing or due to sequencing kits (see reference demonstrating the significance of removing variant blacklists)[65]. Visual inspection of the quality of sequencing reads was performed using The Integrative Genomics Viewer (IGV)[20] software for all prioritized rare and novel variants in order to ensure high quality of retained variants. All the retained variants were manually inspected using scientific literature and genome databases (**Table S2-4**). Variants were classified according to the ACMG guidelines[21].
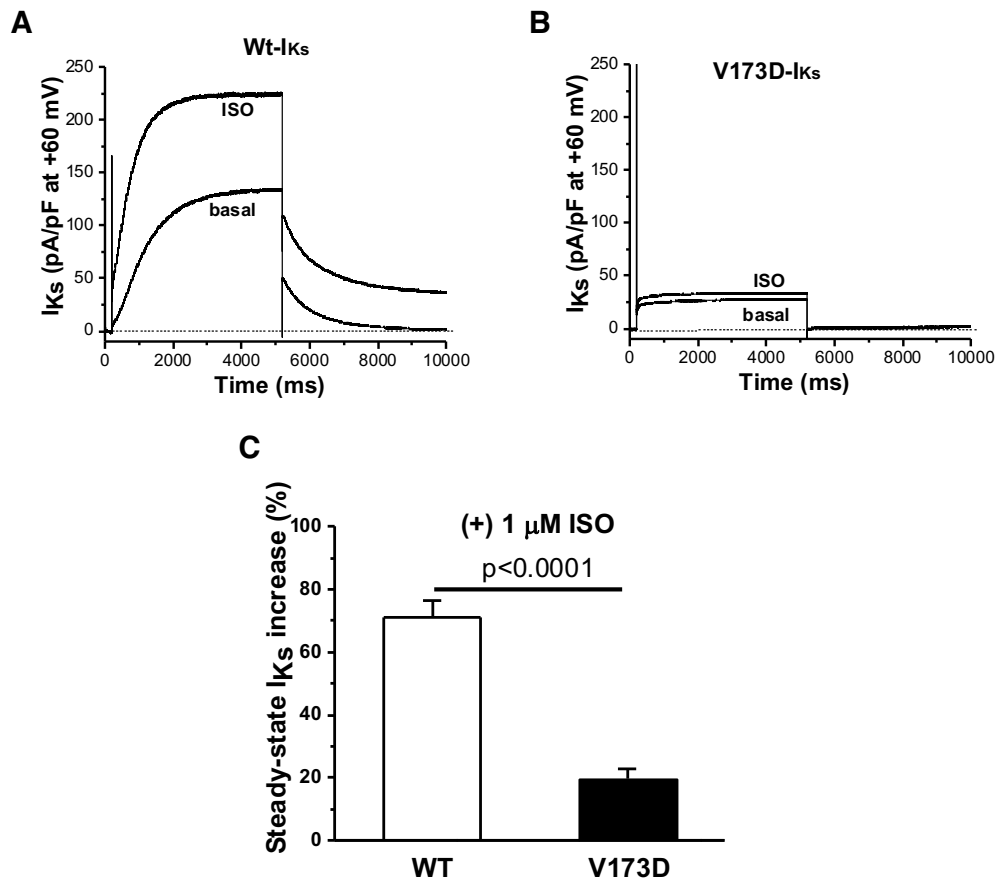
Figure S2. WT- and V173D-I$_{Ks}$ responses to isoproterenol (ISO, 1 μM). Panel A and B show current traces of WT- and V173D-I$_{Ks}$ before and after ISO. Panel C is a summary of ISO-increased steady-state I$_{Ks}$ (%) in WT and V173D by 5-sec pulsing to +60 mV from holding potential of -80 mV (n=6 each).