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Heritability in a *SCN5A*-mutation founder population with increased female susceptibility to non-nocturnal ventricular tachyarrhythmia and sudden cardiac death

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BACKGROUND Heritable cardiac-sodium channel dysfunction is associated with various arrhythmia syndromes, some predisposing to ventricular fibrillation. Phenotypic diversity among carriers of identical-by-descent mutations is often remarkable, suggesting influences of genetic modifiers.

OBJECTIVE The purpose of this study was to identify a unique *SCN5A*-mutation founder population with mixed clinical phenotypes and sudden cardiac death, and to investigate the heritability of electromechanical traits besides the *SCN5A*-mutation effect.

METHODS The 16-generation founder population segregating *SCN5A* c.4850_4852delTCT, p.(Phe1617del), was comprehensively phenotyped. Variance component analysis was used to evaluate the mutation's effects and assess heritability.

RESULTS In 45 p.(Phe1617del) positives, the mutation associated strongly with QTc prolongation (472 ± 60 ms vs 423 ± 35 ms in 26 mutation negatives; $P < .001$; odds ratio for long-QT syndrome 22.4; 95% confidence interval 4.5–224.2; $P < .001$) and electromechanical window (EMW) negativity (−29 ± 47 ms vs 34 ± 26 ms; $P < .001$). Overlapping phenotypes including conduction delay and Brugada syndrome were noted in 19. Polymorphic ventricular tachyarrhythmias occurred mostly in the daytime, after arousal-evoked heart-rate acceleration and repolarization prolongation. Cox proportional hazards regression analysis revealed female gender as an independent risk factor for cardiac events (hazard ratio 5.1; 95% confidence interval 1.6–16.3; $P = .006$). p.(Phe1617del) was an important determinant of $QTc_{baseline}$, QTc_{max}, and EMW, explaining 18%, 28%, and 37%, respectively, of the trait's variance. Significant heritability was observed for PQ interval ($P = .003$) after accounting for the p.(Phe1617del) effect.

CONCLUSION This *SCN5A*-p.(Phe1617del) founder population with phenotypic divergence and overlap reveals long-QT syndrome–related and arousal-evoked ventricular tachyarrhythmias with a female preponderance. Variance component analysis indicates additional genetic variance for PQ interval hidden in the genome, besides a dominant p.(Phe1617del) effect on QTc and EMW.

KEYWORDS Gender differences; Genetics; *SCN5A*; Sudden cardiac death; Ventricular fibrillation

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Introduction

Sudden cardiac death (SCD) accounts for a significant proportion of natural mortality in industrialized societies and imposes a sizable socioeconomic and psychosocial burden. Ventricular fibrillation (VF) is the main cause of SCD and occurs often in the setting of acute coronary events.[1,2] A prominent familial component contributing to the risk of SCD by ischemic VF has been recognized,[3–5]

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but the underlying genetic mechanisms remain largely unclear.

The unraveling of genetic contributors to arrhythmia phenotypes is important for diagnostic purposes, risk stratification, elucidation of molecular pathways, and identification of potential therapeutic targets. However, limited sample sizes of representative populations, heterogeneity of the arrhythmogenic substrate, and difficulty of obtaining phenotype information after cardiac arrest make this a challenging quest. Moreover, prominent phenotypic heterogeneity, including SCD susceptibility, has been observed among affected individuals sharing the same mutation. This variability has been attributed to a low disease penetrance (25% in long-QT syndrome [LQTS][6] and 16% in Brugada syndrome[7]), differential expressivity, and nongenetic factors such as age,[8] gender,[9] and use of medication.[10] One study observed compound heterozygosity in ~8% of LQTS cases.[11] Additionally, common variants in certain genes, such as *SCN5A,*[12] *SCN10A,* and *HEY2,*[13] can contribute to disease penetrance in sick-sinus and Brugada syndrome, which indicates a complex genetic architecture. To investigate such complex genetic factors, family cohorts provide substantial benefits: they are robust to false-positive findings due to population substructure; they typically share a large proportion of environmental factors; and they may be enriched for rare variants that are difficult to study in the general population.

In this study, we investigated a large Dutch-German founder population with excess SCD over multiple generations, segregating an amino-acid deletion in *SCN5A* (c.4850_4852delTCT, exon 28, p.(Phe1617del), rs749697698). Because cardiac differences between Phe1617del carriers appeared prominent, we used variance component analysis to first assess the effects imposed by the known *SCN5A* mutation and then calculate remaining heritabilities of various proarrhythmic phenotypes. Identifying new genetic risk factors can give profound insights into molecular mechanisms, assist in further diagnosis, and yield novel information regarding the broader problem of SCD.

Methods

All participants (≥18 years) provided written informed consent for clinical and genetic analyses. The study protocol was approved by the Medical Research Ethics Committee of Maastricht University Medical Centre. The study complied with the principles of the Declaration of Helsinki and is registered under ClinicalTrials.gov Identifier NCT02014961.

Study population

Between 2006 and 2015, 7 probands presented independently at our Cardiogenetics Outpatient Clinic with QT prolongation, polymorphic ventricular tachycardia (VT), prolonged repolarization–associated torsades de pointes, or SCD of a first-degree relative. After obtaining informed consent, genomic DNA was extracted from peripheral blood. Using Sanger sequencing, the pathogenic *SCN5A* mutation c.4850_4852delTCT, p.(Phe1617del), rs749697698 was identified in all probands on a haplotype that also contained rs1805124 [*SCN5A* c.1673A>G, exon 12, p.(His558Arg)]. One additional variant, rs1805128 in *KCNE1*, c.253G>A, exon 3, p.(Asp85Asn),[14] was found in 2 probands. Both had a prolonged QT interval but no documented VT. The presence of this *KCNE1* variant in the pedigree is unlikely to affect our general results, as its transmission is independent of the *SCN5A*-founder haplotype. No pathogenic mutations were found in *ABCC9*, *AKAP9*, *ANK2*, *CACNA1C*, *CACNA2D1*, *CACNB2*, *CALM1*, *CASQ2*, *CAV3*, *DPP6* (only tested for c.1-340C>T), *GPD1L*, *HCN4*, *KCNE2*, *KCNE3*, *KCNH2*, *KCNJ2*, *KCNJ8*, *KCNQ1*, *LMNA*, *RYR2*, *SCN1B*, *SCN3B*, *SCN5A*, *SNTA1*, *TNNT2*, and *TRDN*.

Relatedness of all Phe1617del probands was ascertained using regional genealogical archives, parish registers, and private databases, and common ancestry was confirmed. Haplotype sharing at 8 microsatellite markers spanning the *SCN5A* locus, 5 upstream (D3S1768, D3S1561, D3S1298, D3S3639, D3S1260) and 3 downstream (D3S1100, D3S2407, D3S3559), confirmed these genealogical relationships. Cascade screening revealed an autosomal dominant inheritance pattern.

Phenotypic characterization

Comprehensive phenotyping of Phe1617del-positive and mutation-negative family members was performed. Syncope, documented VT/VF, aborted cardiac arrest, and/or SCD were scored as cardiac events. Baseline 12-lead ECGs were digitally magnified and analyzed using onscreen calipers. First (≥18 years) and maximal documented PQ and QT intervals were determined. The longest QT interval in lead II or any precordial lead was measured for calculation of the heart rate–corrected QTc according to the Bazett formula. LQTS was diagnosed according to current guidelines.[15] Isorhythmic atrioventricular dissociation was noted as the wandering of sinus P waves in and out of their associated QRS complexes. Overlap was defined as the concomitant presence of the following traits: LQTS, Brugada syndrome, cardiac conduction disease (CCD), or isorhythmic atrioventricular dissociation. Echocardiography and cardiac magnetic resonance imaging were used to determine the presence of structural abnormalities. The electromechanical window (EMW), defined as Q onset to aortic valve closure (QAoC) minus the concomitantly measured QT interval, was calculated during continuous-wave Doppler flow assessment of the aortic valve.[16] Ajmaline provocation testing (1 mg/kg in 10 minutes) was performed to unmask concealed Brugada syndrome. Implantable cardioverter–defibrillator (ICD) readouts were analyzed for arrhythmia characteristics.

Statistical and variance component analyses

The normality of phenotype and residual distributions was assessed by inspection of density plots and by the Shapiro–Wilk test. For continuous traits, mean differences between

subgroups were tested using the Mann–Whitney *U* test. Odds ratios (ORs) comparing the prevalence of dichotomous traits among subgroups were estimated using the Fisher exact test. Cox proportional hazards models were implemented to conduct survival analyses, and testing of the Schoenfeld residuals was performed. Analyses were conducted using the base R package (https://www.R-project.org) and the survival package (https://CRAN.R-project.org/package = survival).

To estimate the effects of the *SCN5A*-p.(Phe1617del) mutation as well as trait heritabilities, a variance component approach, as implemented in the sequential oligogenic linkage analysis routines (SOLAR) software package, was used. Thus, we assessed the narrow-sense heritability estimate (h^2), referred to as "residual heritability," representing the percentage of variance of a quantitative phenotypic trait attributable to additional genetic factors. To satisfy distributional assumptions inherent to this method, phenotypes were regressed on age, sex, and height using linear regression models. The residuals from those models were transformed using the rank-based inverse normal transformation function in GenABEL and used as phenotypes for analysis in models including the *SCN5A* deletion mutation.[17] To correct for multiple comparisons, while accounting for the correlation between phenotypes, the effective number of independent traits was estimated. First, a Spearman correlation matrix was constructed from all analyzed phenotypes in R. Then, matrix spectral decomposition was applied to these correlations using matSpD (http://gump.qimr.edu.au/general/daleN/matSpD/)[18] to derive the number of effective tests (n = 12). The nominal *P* value of .05 was divided by this number, yielding a significance threshold of $P = .004$. From the normalized residuals we computed the effect size (β, in standard deviations (SDs)) attributed to the *SCN5A* mutation, the p.(His558Arg) polymorphism, and the familial component (h^2) by means of a kinship matrix, which was constructed using the pedigree structure.

Results

Genealogy, pedigree structure, and mortality data

Figure 1 illustrates the 16-generation pedigree of the Phe1617del-positive family, which mainly resides in Limburg (The Netherlands) and North Rhine-Westphalia (Germany). Carriers living in Lund (Sweden) and Munich (Germany) had comparable DNA profiles, confirming relatedness, but their lines of descent remained unclear. Genealogical research identified 2 ancestral couples that lived near the river Worm, at the southeastern border of The Netherlands with Germany (hence our designation "Worm Study" to this research), in the 16th century. Ancestral couple A connects 5 probands, and couple B connects 4 (Figure 1). Despite extensive efforts, a single founding couple connecting all probands has not yet been identified. In all likelihood, the *SCN5A* mutation emerged before 1600. Supplementary Figure S1 demonstrates the chronological migration of the mutation from its presumed first occurrence. At present, the total number of known family members exceeds 4,800.

The pedigree depicts interfamilial marriages within the third or fourth degree of consanguinity. A male proband born of consanguineous parents was found to be homozygous for *SCN5A*-p.(Phe1617del) (Figures 1 and 2).

Phenotypic heterogeneity and overlap

Clinical characteristics of the study population are given in Table 1. Four main phenotypes were observed: LQTS, CCD, Brugada syndrome, and isorhythmic atrioventricular dissociation (Figure 2), with striking phenotypic heterogeneity among Phe1617del-positive patients, even between siblings (I–IV in Figure 1).

As illustrated by the color codes in Figure 1, LQTS was the prevailing phenotype, present throughout the pedigree. LQTS was diagnosed in 28 carriers, and a strong association between Phe1617del and risk of LQTS was seen (OR 22.4;

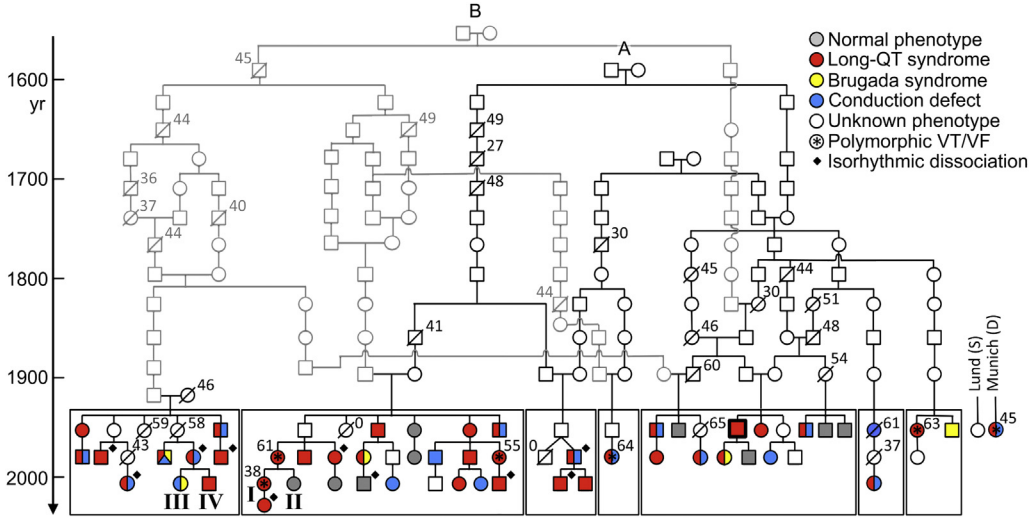

Figure 1 Pedigree of *SCN5A*-p.(Phe1617del)-mutation carriers for 7 family groups. *Black lines* of descent for presumed founder couple A; *gray lines* for couple B. Deaths before age 51 years are noted. *Squares* represent males (homozygous carrier in bold); *circles* represent females. Only carriers are depicted. VF = ventricular fibrillation; VT = ventricular tachycardia.

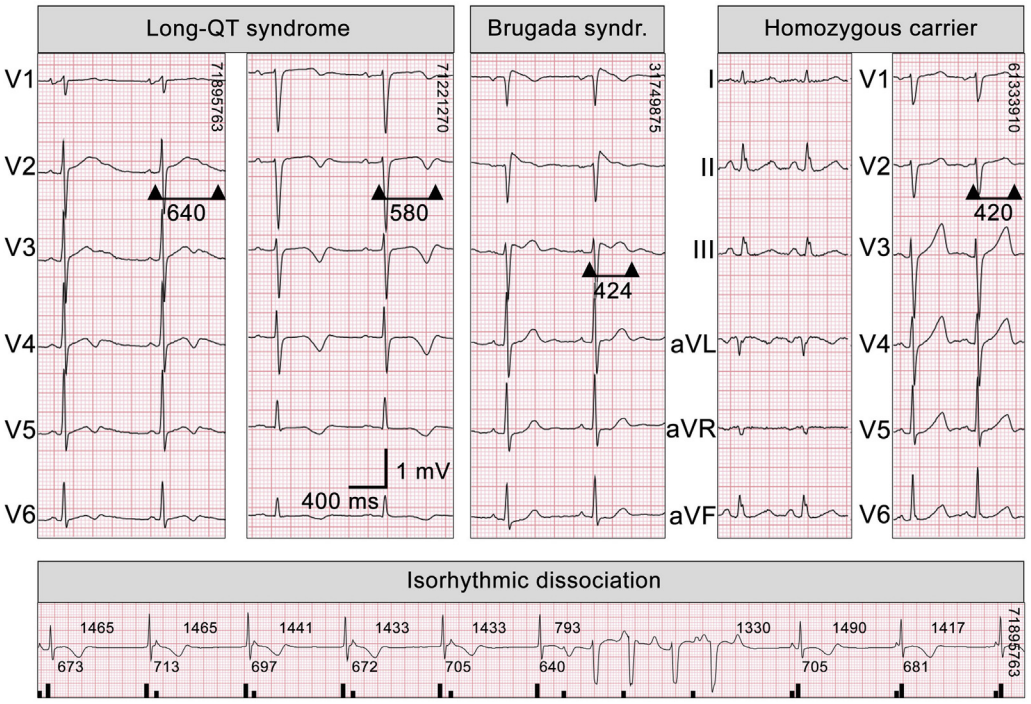

Figure 2 *SCN5A*-p.(Phe1617del)-related electrocardiographic phenotypes. During isorhythmic atrioventricular dissociation, *long bars* and *short bars* indicate QRS and P onset, respectively. *Top numbers* indicate RR intervals; *bottom numbers* indicate QT intervals.

95% confidence interval [CI] 4.5–224.2; $P < .001$). Prolonged repolarization, with a mean difference ($\Delta\mu$) of 48 ms ($P < .001$) compared to noncarriers, was present at a wide variety of heart rates (see Supplementary Figure S2). Phe1617del positives with previous cardiac events exhibited a significantly longer QTc and were more often female compared to event-free carriers (QTc 505 ± 71 ms vs 448 ± 37 ms; $P = .006$; female gender 76% vs 33%; $P = .007$). On echocardiography, QAoCs were significantly shorter ($\Delta\mu = 20$ ms; $P = .028$) despite (simultaneously measured) longer QT intervals, causing more pronounced EMW negativity in mutation positives ($\Delta\mu = -63$ ms) (Table 1). Symptomatic cases had more negative EMWs compared to asymptomatic individuals (−56 ± 60 ms vs −11 ± 26 ms; $P = .008$).[16] *SCN5A*-p.(Phe1617del) carriers frequently exhibited broad-based T-wave inversion or bifid T waves (Figure 2), unlike "classic" LQTS3 repolarization patterns. Interestingly, 2 Phe1617del-negative individuals also exhibited long QT intervals (see Supplementary Figure S3); mutations in known arrhythmia genes were excluded in them.

Whereas baseline PQ and QRS intervals generally remained within normal limits (see Supplementary Figure S2), transient atrioventricular conduction delay was observed in 10 Phe1617del positives, second-degree atrioventricular block in 3, left bundle branch block in 3, and right bundle branch block in 1. A few mutation negatives demonstrated mild forms of CCD. Unique to the Phe1617del-positive cohort, spontaneous coved-type ST-segment elevation fitting with Brugada syndrome (n = 1) (Figure 2) and ajmaline-provoked type 1 Brugada ECG pattern were observed (n = 4) (see Supplementary Figure S4).

A novel phenotype, isorhythmic atrioventricular dissociation, was present in 9 of 45 mutation carriers but was absent in noncarrying relatives ($P_{\text{Fisher}} = .010$) (Figures 2 and 3). It occurred in 3 of 7 family subgroups mainly at night, between 9 PM and 10 AM, at a mean sinus/atrial rate of 41 ± 7 bpm. Besides episodic sinus and atrial bradycardia, no distinct atrial electropathology (such as atrial standstill or atrial fibrillation) was observed. *SCN5A*-related dilated cardiomyopathy was not seen.

The Venn diagram demonstrates that most Phe1617del carriers, notably those with LQTS, exhibited overlap with other phenotypes, namely, CCD, Brugada syndrome, and/or isorhythmic atrioventricular dissociation (n = 19) (Figure 3). In 8 Phe1617del-positive individuals, we could not discern any cardiac abnormalities, despite thorough phenotyping, including ajmaline provocation testing.

Ventricular arrhythmogenesis

In the last 4 decades, 47% of the Phe1617del positives experienced cardiac events (OR 3.8 ; 95% CI 1.1–15.1; $P = .022$), including syncope in 9, nonsustained VT in 3, polymorphic VT/VF in 6 (Figure 1, asterisks), and SCD without arrhythmia documentation in 3. Five ungenotyped first-degree relatives of Phe1617del positives died suddenly. The mean age at which SCD or VT/VF occurred first was 50 ± 10 years. ICD recordings of VT/VF in 3 patients, all females, are depicted in Figure 4. VT/VF episodes required internal/external cardioversion (lower panel), intravenous magnesium and isoproterenol,[19] antitachycardia pacing (middle panel), or recovered spontaneously (upper panel).

Table 1 Clinical characteristics of the study population

	Worm study population			Phe1617del positives		
	Mutation negative	Mutation positive	*P* value	Asymptomatic	Symptomatic	*P* value
No.	26	45		24	21	
Age (yrs)	51 ± 18	49 ± 16	.68	49 ± 17	50 ± 16	.86
Female	16 (62)	24 (53)	.81	8 (33)	16 (76)	.007
Height (cm)	171 ± 13	174 ± 11	.34	176 ± 9	170 ± 12	.036
Weight (kg)	73 ± 15	73 ± 13	.71	75 ± 11	68 ± 15	.048
Cardiac events	5 (19)	21 (47)	.022	0	21	
Beta-blocker	8 (31)	13 (29)	.78	5 (24)	8 (38)	.18
Cardiac phenotype						
Normal	15 (58)	8 (18)	.003	6 (25)	2 (10)	.43
LQTS	2 (8)	28 (62)	<.001	14 (58)	14 (67)	.18
CCD	10 (38)	15 (33)	1	8 (33)	7 (33)	.75
Brugada syndrome	0 (0)	5 (11)	.15	3 (13)	2 (10)	1
Isorhythmic dissociation	0 (0)	9 (20)	.010	5 (21)	4 (19)	1
Overlap	1 (4)	19 (42)	<.001	9 (38)	10 (48)	.34
Unknown		4 (9)			4 (19)	
Electrocardiography						
PQ (ms)	164 ± 31	161 ± 25	.80	166 ± 24	153 ± 24	.076
PQ_{max} (ms)	176 ± 37	186 ± 41	.29	193 ± 48	177 ± 28	.31
RR (ms)	934 ± 150	910 ± 181	.57	909 ± 192	912 ± 170	.91
QRS (ms)	100 ± 16	93 ± 14	.12	92 ± 8	96 ± 19	.94
QT (ms)	408 ± 42	450 ± 74	.008	424 ± 49	487 ± 88	.03
QTc (ms)	423 ± 35	472 ± 60	<.001	448 ± 37	505 ± 71	.006
QTc_{max} (ms)	459 ± 46	523 ± 69	<.001	499 ± 46	557 ± 83	.043
Echocardiography						
LVEF (%)	60 ± 6	62 ± 5	.11	62 ± 5	61 ± 5	.39
RVEDD (mm)	34 ± 6	34 ± 4	.71	33 ± 8	35 ± 4	.60
QAoC (ms)	420 ± 31	400 ± 32	.029	399 ± 25	402 ± 42	.68
RR (ms)	957 ± 171	900 ± 123	.14	897 ± 110	906 ± 146	.84
QT (ms)	386 ± 38	428 ± 54	.001	410 ± 28	456 ± 72	.037
EMW (ms)	34 ± 26	−29 ± 47	<.001	−11 ± 26	−56 ± 60	.008

Values are given as mean ± SD or n (%) unless otherwise indicated.

For continuous traits, mean differences were tested using the Mann–Whitney *U* test. Differences in prevalence of dichotomous traits were compared using the Fisher exact test. Four phenotypes were missing because of sudden cardiac death.

CCD = cardiac conduction disease; EMW = electromechanical window; LVEF = left ventricular ejection fraction; QAoC = time interval between onset QRS and aortic valve closure; LQTS = long-QT syndrome; RVEDD = right ventricular end-diastolic diameter.

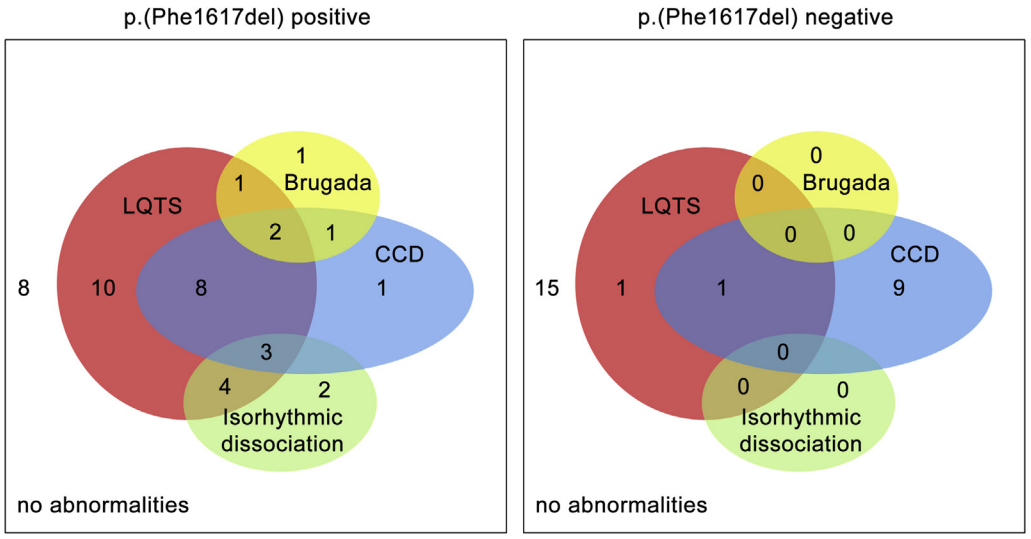

Figure 3 Venn diagram illustrating cardiac phenotypes and their overlap in the presence or absence of *SCN5A*-p.(Phe1617del). In both panels, some numbers are missing because of incomplete cardiac recordings. CCD = cardiac conduction disease; LQTS = long-QT syndrome.

Beta-blocker treatment and high-rate atrial pacing did not prevent VT/VF in 2 patients. Ranolazine, however, reduced ventricular ectopy substantially. These life-threatening arrhythmias were found to occur only during daytime, between 9 AM and 9 PM, in line with the daytime occurrence of SCD in other confirmed or obligatory Phe1617del positives in this population. Proarrhythmic triggers included auditory stimuli (phone ringing) (Figure 4), strong emotions, exercise (skiing), a period of excessive dieting (lower panel), and events in early postoperative and postpartum periods. VT occurred rarely during rest or sleep. Often, sinus rate acceleration with concomitant repolarization prolongation preceded premature ventricular complexes and polymorphic VT, as exemplified in Figure 4. Torsades de pointes–like VT typically initiated after short–long–short RR intervals or could be non–pause dependent.

We found a 15:1 female-to-male ratio of cardiac events ($P = .007$) despite a similar mean QTc and EMW for both sexes (QTc 470 ± 55 ms vs 474 ± 66 ms; $P = .83$; EMW −20 ± 43 ms and −36 ± 52 ms; $P = .31$). Figure 5 shows survival curves estimated using Cox proportional hazards models for event-free survival adjusted for gender, LQTS, EMW negativity (<−62 ms[16]), and beta-blocker therapy. The survival curves are conditional, based on the covariates. Hazard ratios (HRs) indicated an independently increased risk for females (HR 5.1; 95% CI 1.6–16.3; $P = .006$) and for individuals with substantial EMW negativity (HR 4.3; 95% CI 1.2–15.6; $P = .024$). Testing of the Schoenfeld residuals demonstrated that the proportionality assumption held ($P = .42$). EMW negativity enabled risk stratification in male ($P = .004$), but not in female Phe1617del carriers ($P = .15$).

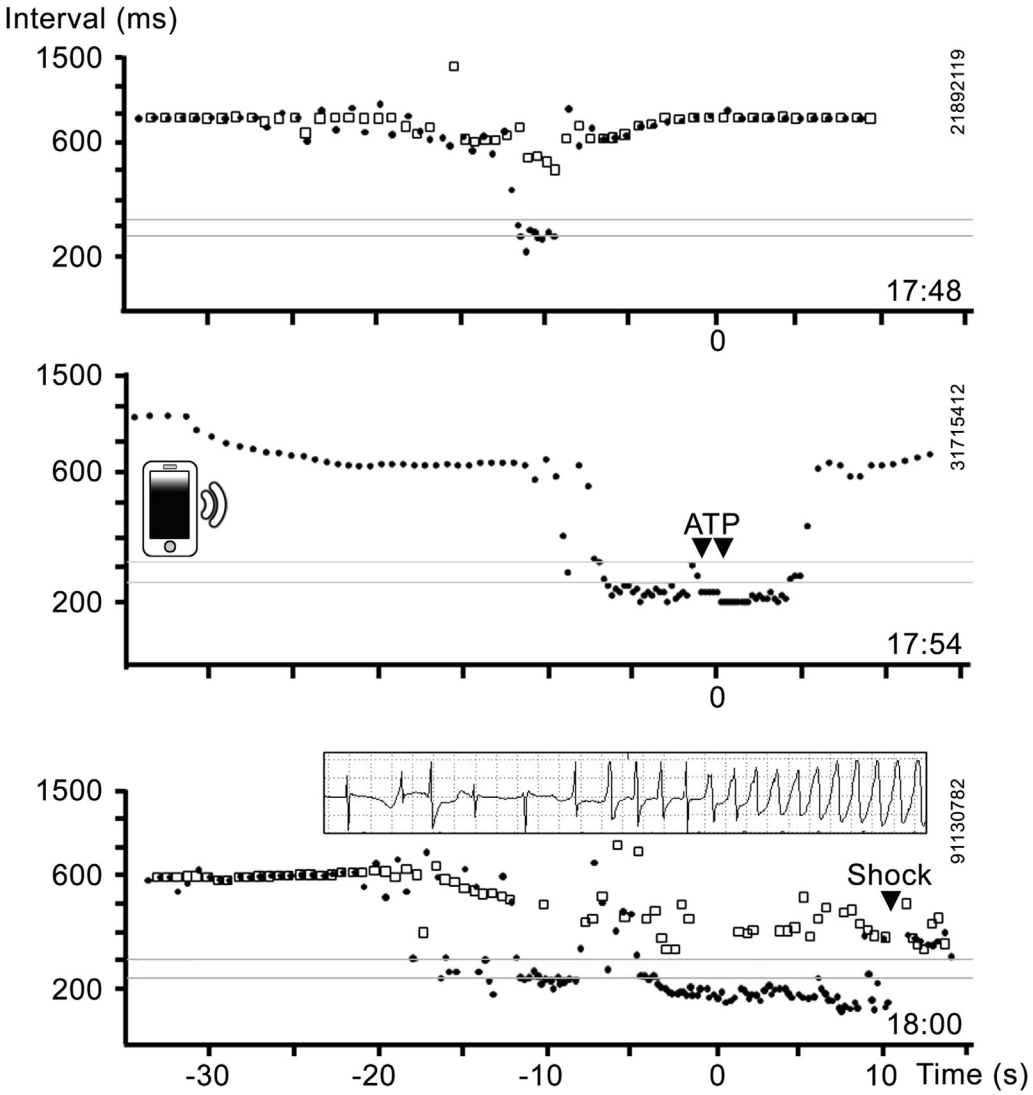

Figure 4 Implantable cardioverter–defibrillator recordings of polymorphic ventricular tachycardia in 3 females. **Top:** Spontaneous recovery. **Middle:** Cardioversion by antitachycardia pacing. **Bottom:** Cardioversion by shock therapy. Time (h:min) indicates ventricular tachycardia/ventricular fibrillation occurrence. *Squares* indicate AA intervals; *circles* indicate VV intervals.

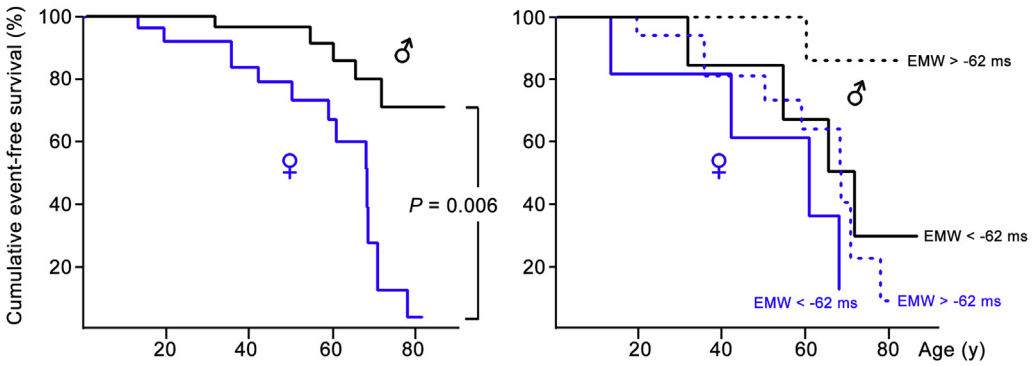

Figure 5 Cox proportional hazards curves for event-free survival adjusted for beta-blocker therapy, gender, EMW (<−62 ms or > −62 ms), and long-QT syndrome; and stratified by gender and EMW negativity. EMW = electromechanical window.

Phe1617del-related effects and residual heritability

Analyses conducted in SOLAR, to properly account for the pedigree structure, recapitulated the strength of the associations between p.(Phe1617del) and $QTc_{baseline}$, QTc_{max}, and EMW (Table 2). Effect sizes were large, on the order of magnitude of 1 SD for $QTc_{baseline}$ [β (SE) = 0.91 (0.23)], QTc_{max} [1.11 (0.22)], and EMW [−1.26 (0.19)], explaining 18%, 28% and 37% of the trait's variance, respectively. These estimates corresponded well with the observed mean differences. Nominal associations were seen for QRS, QT, and QAoC.

Heritability estimates for baseline PQ interval [h^2 (SE) = 0.68 (0.25); P = .003] showed that additive genetic effects accounted for a substantial proportion of PQ variation, independent of p.(Phe1617del). Nominally significant heritabilities were observed for PQ_{max} [(h^2 (SE) = 0.57 (0.27)], QT_{max} [0.43 (0.27)], and EMW [0.79 (0.29)].

Table 2 Residual heritabilities and effects of *SCN5A*-p.(Phe1617del)

			SCN5A	
	h^2 (SE)	*P* value	β (SE)	*P* value
Electrocardiography				
PQ	0.68 (0.25)	.003	−0.01 (0.24)	.98
PQ_{max}	0.57 (0.27)	.010	0.47 (0.25)	.056
RR	0.00 (NE)	.50	−0.17 (0.27)	.53
QRS	0.19 (0.23)	.17	−0.52 (0.25)	.040
QT	0.43 (0.27)	.049	0.56 (0.24)	.021
QTc	0.23 (0.37)	.28	0.91 (0.23)	<.001
QTc_{max}	0.35 (0.63)	.36	1.11 (0.22)	<.001
Echocardiography				
LVEF	0.59 (0.37)	.19	0.28 (0.26)	.27
RVEDD	0.08 (0.19)	.31	0.09 (0.27)	.75
QAoC	0.00 (NE)	.50	−0.51 (0.25)	.039
QT	0.55 (0.43)	.10	0.94 (0.23)	<.001
EMW	0.79 (0.29)	.020	−1.26 (0.19)	<.001

A variance component approach as implemented in SOLAR (sequential oligogenic linkage analysis routines) was used.

β = effect size in standard deviations for *SCN5A*-p.(Phe1617del) carriership; h^2 = narrow-sense heritability; NE = not estimable; SE = standard error; other abbreviations as in Table 1.

The additional inclusion of *SCN5A*-p.(His558Arg), rs1805124, did not significantly alter the results and provided no evidence of an independent effect on any of the traits studied (all P >.10), with the possible exception of PQ_{max} (P = .064). Although the p.(His558Arg) polymorphism was in Hardy–Weinberg proportions in Phe1617del-negative family members (P = .10), there was a significant enrichment for the R allele in mutant carriers compared to the EUR samples from the 1000 Genomes Project Phase 3 (35% vs 22%; P_{Fisher} <.001).

Discussion

Unique founder population

This *SCN5A*-p.(Phe1617del) founder population exhibited divergent and overlapping cardiac phenotypes, including LQTS, CCD, Brugada syndrome, and isorhythmic atrioventricular dissociation. Female carriers had a dominant susceptibility to ventricular arrhythmia and SCD, despite a QTc similar to that of males. Large p.(Phe1617del) effect sizes were found for QTc prolongation and EMW negativity. After accounting for the effects of the *SCN5A* deletion mutation, significant heritability was identified for PQ interval, indicating the influence of additional genetic factor(s) on atrioventricular conduction.

Phenotypic variability in cardiac sodium channelopathies

Since the first localization of the *SCN5A* locus to chromosome 3q21-24 and the description of the *SCN5A* mutation p.(KPQ1505-1507del) in 1995,[20] multiple pathogenic *SCN5A* mutations have been implicated in LQTS3, Brugada syndrome, idiopathic VF, CCD, sinus node dysfunction, atrial fibrillation, and dilated cardiomyopathy.[21] In the present study, LQTS was the most prevalent phenotype of Phe1617del-positive individuals and possibly the result of an I_{Na} gain-of-function defect. Phenotypes reminiscent of I_{Na} loss-of-function, such as Brugada syndrome and CCD, were also present but less prominent. Previous patch-clamp experiments of Phe1617del-transfected HEK cells demonstrated a 7.0-mV negative shift of steady-state channel availability and impaired recovery from inactivation,

contributing to reduced peak I_{Na} density.[22] Those investigators found an increased late/peak I_{Na} ratio at positive command potentials, suggesting gain of function for part of the I_{Na} I–V curve. The presence of phenotypic divergence and phenotype-positive genotype-negative individuals alludes to the contribution of additional genetic variants. Furthermore, *SCN5A*-p.(Phe1617del) was identified in 2 individuals of different ethnicity (gnomAD database) who had LQTS and syncope without LQTS/Brugada syndrome characteristics. SOLAR analysis revealed a large effect of the mutation on repolarization prolongation, which is underscored by the segregation pattern. Combined, these results indicate that a definitive or monogenic causality cannot be assumed, and this has instigated subsequent studies into the area of complex genetics.

Variance component analysis and heritability beyond *SCN5A*-p.(Phe1617del)

Although the presence of *SCN5A*-p.(Phe1617del) associated strongly with QTc, QAoC, and EMW, we did find evidence of other genetic influences on PQ interval, and possibly QT and EMW. PQ heritability (0.68) was higher than in a general population family-based study (0.40) in The Netherlands,[23] indicating strong genetic control in our pedigree. The *SCN5A* polymorphism p.(His558Arg) present in ~22% of EUR samples from the 1000 Genomes Project (http://www.internationalgenome.org) was significantly enriched in this population (35%). Variance component analysis incorporating p.(His558Arg) provided no evidence of an independent effect on any of the traits investigated, although enrichment for the p.(Arg558) allele supported the notion of population isolate effects, particularly founder effects and genetic drift. Functional variants in other genes or regulatory elements on chromosome 3, such as *SCN10A*,[24] could also modulate regional *SCN5A* expression. Moreover, genetic modifiers on different chromosomes may be important. These aspects will be investigated in downstream high-density single nucleotide polymorphism genotyping and/or exome sequencing (Figure 6).

Arrhythmia susceptibility and female preponderance

Contrary to previously reported *SCN5A*-related arrhythmia features,[21] we mainly observed stress-related, non-nocturnal VT/VF provoking cardiac arrest/SCD in our *SCN5A*-p.(Phe1617del) population. Stress- and arousal-evoked heart rate acceleration and repolarization prolongation often preceded the life-threatening arrhythmias. These patterns

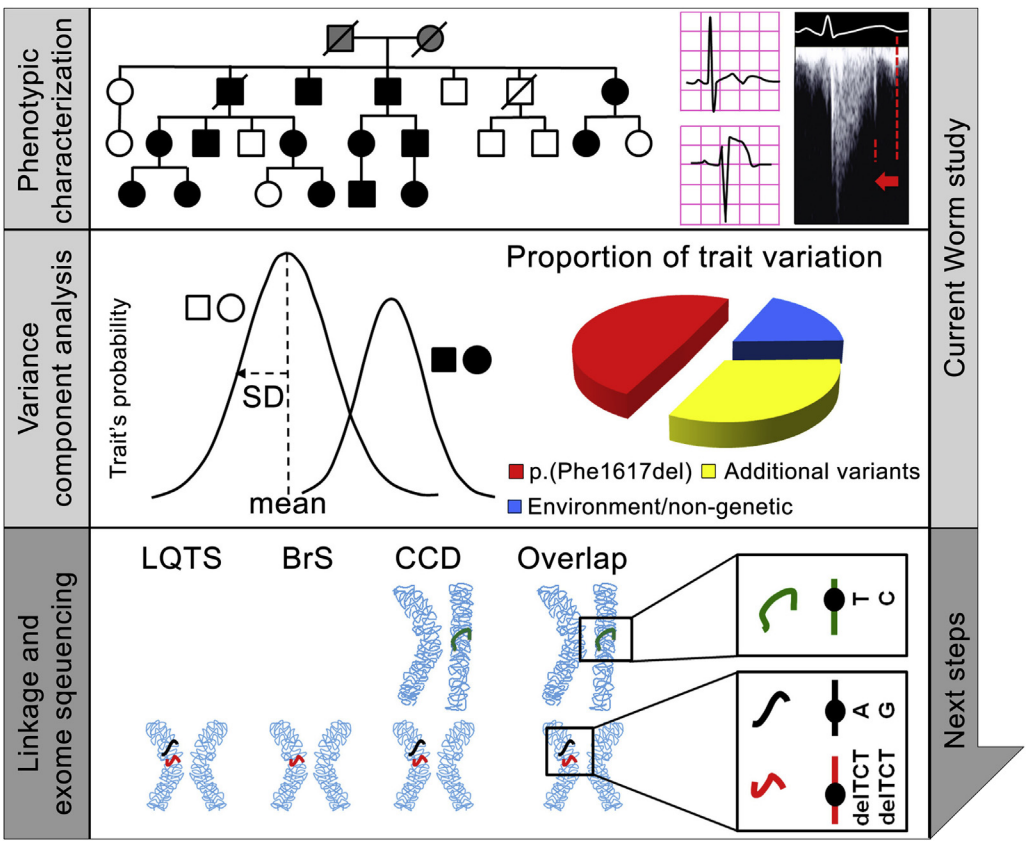

Figure 6 In-depth cardiac phenotyping coupled with variance component analysis explains electromechanical trait variation. The proportion of mutant and additional genetic variant (narrow-sense heritability)-imposed trait variation is estimated using SOLAR (sequential oligogenic linkage analysis routines). As a perspective, subsequent studies will encompass linkage analysis and next-generation sequencing. *Boxes* indicate males; *circles* indicate females. *Open symbols* indicate Phe1617del negatives; *filled symbols* indicate Phe1617del positives. BrS = Brugada syndrome; CCD = cardiac conduction disease; LQTS = long-QT syndrome; SD = standard deviation.

suggest a proarrhythmic role for enhanced sympathetic activity to fuel electrical instability, which is uncommon for sodium channelopathies. Second, unlike reported sex-specific risk patterns,[9,25] we found that women with *SCN5A*-p.(Phe1617del) experienced major cardiac events more frequently than men. Fifty percent had (one or more) syncope or VT/VF/SCD before the age of 45. This female arrhythmia susceptibility may be evoked by altered myocardial ion-channel expression through modulation by (non)gonadal hormones.[26] Also, gene–gender interaction may play a role.

Conclusion

This unique *SCN5A*-p.(Phe1617del) founder population with phenotypic heterogeneity and overlap syndrome reveals a large impact of p.(Phe1617del) on QTc, QAoC, and EMW. SCD and polymorphic VT/VF occur predominantly in females, during daytime, and often after arousal-evoked heart-rate acceleration and repolarization prolongation. This suggests sympathetic arrhythmia triggering, unlike what is known for other sodium channelopathies. Substantial genetic variance for PQ (and possibly QT) after accounting for p.(Phe1617del) may contribute to the phenotypic diversity. Our approach of synergizing in-depth cardiac phenotyping with statistical genetics reveals crucial information on the electromechanical traits of this population and paves the way for linkage analysis and exome sequencing to pinpoint genetic variants associated with the arrhythmia syndrome.

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Appendix

Supplementary data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.hrthm.2017.07.036.

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