Further Insights in the Most Common *SCN5A* Mutation Causing Overlapping Phenotype of Long QT Syndrome, Brugada Syndrome, and Conduction Defect

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Background—Phenotypic overlap of type 3 long QT syndrome (LQT3), Brugada syndrome (BrS), cardiac conduction disease (CCD), and sinus node dysfunction (SND) is observed with *SCN5A* mutations. *SCN5A*-E1784K is the most common mutation associated with BrS and LQTS3. The present study examines the genotype–phenotype relationship in a large family carrying *SCN5A*-E1784K and *SCN5A*-H558R polymorphism.

Methods and Results—Clinical work-up, follow-up, and genetic analysis were performed in 35 family members. Seventeen were *SCN5A*-E1784K positive. They also displayed QTc prolongation, and either BrS, CCD, or both. One carrier exhibited SND. The presence of *SCN5A*-H558R did not significantly alter the phenotype of *SCN5A*-E1784K carriers. Fourteen *SCN5A*-E1784K patients underwent implantable cardioverter-defibrillator (ICD) implantation; 4 developed VF and received appropriate ICD shocks after 8 ± 3 months of follow-up. One patient without ICD also developed VF after 6.7 years. These 5 cases carried both *SCN5A*-E1784K and *SCN5A*-H558R. Functional characterization was achieved by expressing *SCN5A* variants in TSA201 cells. Peak ($I_{Na,P}$) or late ($I_{Na,L}$) sodium currents were recorded using whole-cell patch-clamp techniques. Co-expression of *SCN5A*-E1784K and *SCN5A*-WT reduced $I_{Na,P}$ to 70.03% of WT, shifted steady-state inactivation by -11.03 mV, and increased $I_{Na,L}$ from 0.14% to 1.86% of $I_{Na,P}$. Similar changes were observed when *SCN5A*-E1784K was co-expressed with *SCN5A*-H558R.

Conclusions—We demonstrate a strong genotype-phenotype correlation with complete penetrance for BrS, LQTS, or CCD in the largest family harboring *SCN5A*-E1784K mutation described so far. Phenotype of LQTS is present during all decades of life, whereas CCD develops with increasing age. Phenotypic overlap may explain the high event rate in carriers. (*J Am Heart Assoc*. 2016;5:e003379 doi: 10.1161/JAHA.116.003379)

Mutations in the *SCN5A* gene have been associated with long QT syndrome (LQTS) type 3, Brugada syndrome (BrS), sick sinus syndrome, and cardiac conduction disease

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(CCD).[1–3] Some of these mutations can cause an overlapping electrocardiographic phenotype.[4,5] *SCN5A*-E1784K was first described in clinical cases presenting with mild bradycardia, LQTS, and sudden cardiac death (SCD).[6] Recently, this mutation was associated with the phenotype of BrS, LQTS, and sick sinus syndrome. The *SCN5A*-E1784K mutation has been recognized as the most common LQT3 mutation and accounts for up to 34% of LQT3 cases.[5] *In vitro* studies suggest that this common *SCN5A* mutation causes the mixed phenotype by enhanced sodium channel inactivation, a negative shift of steady-state sodium channel inactivation, and enhanced tonic block in response to sodium channel blockers.[5]

Polymorphisms have been shown to modulate *SCN5A* mutations in the sense of either aggravation of the channelopathy or rescue of the pathophysiologic effect of the mutation.[7–10] *SCN5A*-H558R is a common polymorphism and has been shown to restore normal sodium channel function in the case of specific mutations and to enhance sodium channel dysfunction in others.[8,11,12]

The objective of the present study was to elucidate clinical spectrum and electrocardiographic phenotype in a large European family with familial SCD and carrying the *SCN5A*-E1784K mutation and the common polymorphism *SCN5A*-H558R. Furthermore, the electrophysiologic effects of the *SCN5A*-H558R polymorphism on the *SCN5A*-E1784K mutation were studied both *in vitro* and *in vivo*.

Methods

Clinical Analysis

The European family studied consisted of 76 family members (Figure 1). All members were encouraged to undergo a complete clinical work-up and follow-up. The work-up included medical and family history, physical examination, 12-lead baseline and stress ECG, and echocardiogram. ECGs were analyzed by 2 independent cardiologists blended to genotype status. In case of abnormal findings, invasive cardiologic investigations were performed. All members were invited to participate in an ajmaline challenge test and genetic screening for causative mutations. The clinical and genetic studies were approved by the local human ethics committees and performed after written informed consent was obtained from all participants. All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Genetic Analysis

Genomic DNA was prepared from peripheral blood lymphocytes of the patient. Genomic DNA was extracted from peripheral blood lymphocytes using a commercial kit (Gentra System; Puregene, Valencia, CA). All exons and intron borders of the susceptibility genes (*SCN5A, SCN1-4B, GPD1L, CACNA1C, CACNB2b, CACNA2D1, KCNE3-5, KCNJ8, ABCC9*), including the alternative splice variants, were amplified and analyzed by direct sequencing. Polymerase chain reaction products were purified with a commercial enzyme (ExoSAP-IT; USB, Cleveland, OH) and directly sequenced from both directions using an Applied Biosystems 3100 Genetic Analyzer (Applied Biosystem, Foster City, CA). Comprehensive open-reading frame/splice site mutational analysis was then performed.

Cellular Electrophysiologic Analysis

The appropriate nucleotide changes were engineered into human cardiac voltage-dependent sodium channel *SCN5A*/hNav1.5 in the pcDNA3.1 vector (Invitrogen, Carlsbad, CA) as
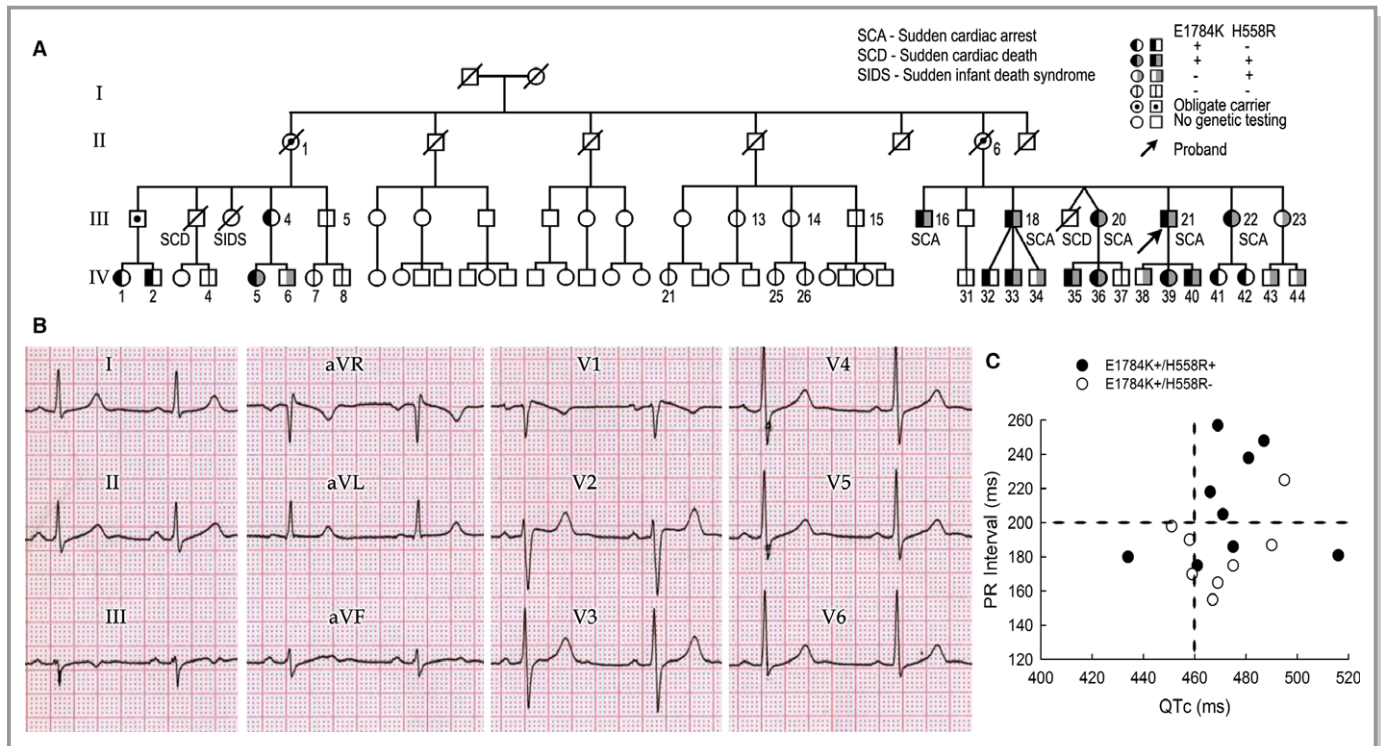
Figure 1. Clinical summary of the affected family. A, Pedigree of the family. B, Baseline ECG of the index patient (proband), who is positive for *SCN5A*-E1784K and H558R. C, PR interval vs QTc among *SCN5A*-E1784K carriers. Upper limit of normal values is indicated by dotted lines.

reported previously.[13,14] Wild-type (WT) and mutant channels were expressed transiently in TSA201 cells for functional study.[13] The WT *SCN1B* cloned in pRC-CMV were co-expressed in each experimental group. CD_8 beads were used for identification.

Macroscopic I_{Na} was measured using a standard whole-cell patch clamp method at a temperature of 22°C to 24°C. The extracellular (bath) solution contained (in mmol/L): 140 NaCl, 5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 2.8 Na acetate, 10 HEPES, and 10 glucose (pH 7.3 with NaOH). Tetraethylammonium (5 mmol/L) was added to the buffer. The pipette solution contained (in mmol/L): 5 NaCl, 5 KCl, 130 CsF, 1.0 $MgCl_2$, 5 EGTA, and 10 HEPES (pH 7.2 with CsOH). Pipettes had resistances between 0.8 and 2.8 MΩ when filled with recording solution. The data were acquired using pClamp 9.2 (Axon Instruments Inc., Union City, CA) and analyzed using Clampfit (Axon Instruments Inc.). The standard voltage clamp protocols and other details are presented with the data and as described in detail previously.[15] Late I_{Na} was recorded in bath solution without and with 25 μmol/L tetrodotoxin. To ensure the accuracy of the value of late I_{Na}, we only included cells with peak $I_{Na} > 1$nA.

Statistical Analysis

Clinical and electrophysiological data were presented as mean±SD and mean±SE, respectively. Before choosing the appropriate statistical test, we checked for normality (Kolmogorov–Smirnov test) and assumption of equal variance (Levene's test). In case of a non-normality or unequal variance the Wilcoxon signed-rank test, the Mann–Whitney *U* test, and the Kruskal–Wallis test were used. Continuous variables among multiple subgroups were analyzed by ANOVA coupled with a Student-Newman-Keuls (SNK) test for electrophysiological study. Chi-square test and Fisher's exact test were used for comparison of categorical variables. Kaplan–Meier survival curves were generated to compare the outcome according to the underlying genotype. Receiver operating characteristic curves were used to analyze ECG parameters in predicting a positive genotype with the corresponding sensitivity and specificity. A $P < 0.05$ was considered statistically significant. All calculations were performed using SPSS 21 (SPSS Inc, Chicago, IL).

Results

Clinical Analysis

The proband (III,21), a 47-year-old male of European descent, presented with aborted SCD (or sudden cardiac arrest [SCA]) due to ventricular fibrillation (VF). His basal ECG showed QTc prolongation of 465 ms, first-degree atrioventricular block (AVB), and ST elevation in the right precordial leads with a type-I and type-II Brugada pattern (Figure 1B). He received an implantable cardioverter-defibrillator (ICD).

The family consisted of 76 family members (Figure 1A). The family history revealed aborted SCA of proband's elder brother (III,18) 3 years ago. The brother exhibited sinus bradycardia, first-degree AVB (PR, 225 ms), and QTc interval of 469 ms without ST-segment elevation in the right precordial leads. He received an ICD and subsequently experienced 4 appropriate ICD shocks due to VF. Furthermore, 3 other family members suffered SCD at the age of 1 year (III,3), 22 years (III,19), and 45 years of age (III,2).

Of the 76 family members, 28 refused genetic analysis and 13 patients died before they could be evaluated. A total of 35 family members (19 males, mean age 29.8±15.9 years) were clinically and genetically evaluated. None of them received Class III anti-arrhythmic drug as medication.

Genetic Analysis

Of the 35 genotyped family members, 17 (8 males/47%; mean age 30.6±15.5 years) were carriers of the *SCN5A*-E1784K mutation. Eighteen members (11 males/61%; mean age 29±16.7 years) were negative for the mutation. No significant differences with respect to age and sex were observed.

The *SCN5A*-H558R polymorphism was identified in a total of 18 members. Nine patients (5 males, mean age 35.1±16.3 years) were positive for both *SCN5A*-E1784K mutation and *SCN5A*-H558R polymorphism, whereas 8 patients (3 males; mean age 25.7±13.9 years) carried only the mutation, but not the polymorphism. Common single nucleotide polymorphisms, V1869M (22.9%) and L1868P (33.1%) in *CACNA1c*, were also found in the proband, which have never been reported as disease-causing or disease-modulating variants.

Out of 18 patients negative for *SCN5A*-E1784K, 9 patients (7 males/78%; mean age 17.6±9.4 years) were carriers of H558R polymorphism. Nine members (4 males/44%; mean age 40.5±14.7 years) were neither carrier of the mutation nor the polymorphism. Patients carrying the polymorphism without mutation were significantly younger than family members negative for both ($P = 0.002$).

Electrocardiographic Analysis

SCN5A-E1784K positive *versus* SCN5A-E1784K negative

Electrocardiographic measurements of *SCN5A*-E1784K carriers *versus* noncarriers are shown in Table 1. Patients positive for *SCN5A*-E1784K exhibited significantly longer PR interval, QRS duration, and QTc interval compared to noncarriers ($P < 0.0001$, $P = 0.03$, $P < 0.0001$, respectively). Ajmaline

Table 1. Electrocardiographic Parameters of *SCN5A*-E1784K Carriers *Versus* Noncarriers

	E1784K+ n=17	E1784K− n=18	*P* Value
RR, ms	920±258	865±168	0.46
PR, ms	196±31	162±17	<0.0001
QRS, ms	100±20	87±13	0.03
QTc, ms	467±16	408±17	<0.0001
ST elevation V1, mV	0.04±0.07	0.01±0.03	0.11
ST elevation V2, mV	0.04±0.07	0.01±0.03	0.08
ST elevation V3, mV	0.02±0.05	0.04±0.02	0.2
Brugada type II/III	7	0	0.008
Ajmaline positive	13/15 (87%)*	0/11 (0%)	<0.0001

*Two had to be stopped due to QRS widening >130% and the occurrence of premature ventricular contractions.

challenge was positive in 87% of patients positive for *SCN5A*-E1784K. In 2 patients, ajmaline administration had to be stopped due to QRS widening >130% and the occurrence of premature ventricular contractions (PVCs). The response to intravenous ajmaline was negative in all *SCN5A*-E1784K noncarriers.

In the receiver operating characteristic analysis, QRS interval was not found to be predictive for the identification of an *SCN5A*-E1784K carrier (area under the curve, 0.692). There was good correlation between PR interval and *SCN5A*-E1784K mutation carrier (area under the curve 0.842). Using a PR interval of 176 ms, sensitivity and specificity for identification of a *SCN5A*-E1784K carrier was 76.5% and 72.2%. The best correlation was found for QTc interval. The cut-off value of QTc=445 ms identified an *SCN5A*-E1784K mutation carrier with a sensitivity of 88% and a specificity of 100% (area under the curve 0.993, Figure 2A).

SCN5A-E1784K and *SCN5A*-H558R

Electrocardiographic parameters with respect to mutation and polymorphism are represented in Table 2 and Figure 1C. There were no significant differences within *SCN5A*-E1784K mutation carriers positive or negative for *SCN5A*-H558R. Carriers with the mutation and polymorphism exhibited a Brugada saddle-back type ECG (55%) more often than patients without the polymorphism (25%). The response to ajmaline administration was positive in 75% (E1784K+/H558R+) and 100% (E1784K+/H558R−), respectively. QRS widening over 130% and PVCs during ajmaline administration occurred in 2 patients with E1784K+/H558R+, which led to the interruption of the test.

In patients negative for *SCN5A*-E1784K mutation, carriers of the polymorphism had a significantly shorter PR interval

and QRS duration (*P*=0.04 and 0.005, respectively). However, patients positive for the polymorphism were significantly younger than patients without the polymorphism (17.6±9.4 *versus* 40.5±14.7 y/o; *P*=0.002). In all patients without mutation, ajmaline challenge tests were negative.

Age and mutation

The impact of age on the electrocardiographic presentation of the *SCN5A*-E1784K carriers *versus* noncarriers is presented in Table 3. Patients were divided in subgroups according to age (age <40 years [mean age 20.4 years] and ≥40 years [mean age 49.3 years]) and presence or absence of *SCN5A*-E1784K. Among patients positive for the *SCN5A*-E1784K mutation, RR-interval, PR interval, and QRS duration were significantly longer in patients ≥40 years of age (*P*=0.007, 0.0001, 0.04). All mutation carriers ≥40 years exhibited a first-degree AVB with a mean PR interval of 231±19 ms. QTc interval was not significantly different between young (<40 years of age) and old (≥40 years of age) mutation carriers (465±15 ms *versus* 470±19 ms; *P*=0.55). In noncarriers there were no significant differences between patients in the subgroups <40 and ≥40 years, although an increase of PR interval and QRS duration was observed.

In comparison of patients <40 years with respect to the absence or presence of *SCN5A*-E1784K, significant differences were found for PR interval and QTc interval. Mutation carriers <40 years had significantly longer PR and QTc intervals compared to noncarriers at the same age (*P*=0.03 and 0.0001). There was a trend of longer QRS duration in mutation carriers (*P*=0.07). In older patients (≥40 years), mutation carriers exhibited significantly longer PR-, QTc interval, and maximal ST elevation in the right precordial leads (*P*=0.001, 0.001, and 0.032, respectively). In addition, when *SCN5A*-H558R was taken into consideration, there is no difference among those with *SCN5A*-E1784K−, no matter in which age subgroup (<40 or ≥40 years, Table 4). When comparing electrocardiographic parameters of *SCN5A*-E1784K+ carriers, the presence or absence of *SCN5A*-H558R also had a similar distribution with age, except the *SCN5A*-H558R+ group showed a lower heart rate in the older-age subgroup (≥40 years), and the *SCN5A*-H558R− group showed a higher ST elevation in the younger-age subgroup (<40 years).

Patient II,6, the mother of the proband, was an obligate mutation carrier. Repetitive ECG recordings at the age of 64, 75, 77, 78, and 81 were available (Figure 3). She developed progressive conduction disease with increasing age. At the age of 75, the patient showed first-degree AVB and 3 years later second-degree AVB. Consecutively, a DDD pacemaker was implanted due to symptomatic bradycardia due to sinus node dysfunction (SND) and second-degree AVB. Furthermore, intermittent ST-segment elevation in lead V1 (typical saddle-back type for the BrS), was visible (Figure 3B).
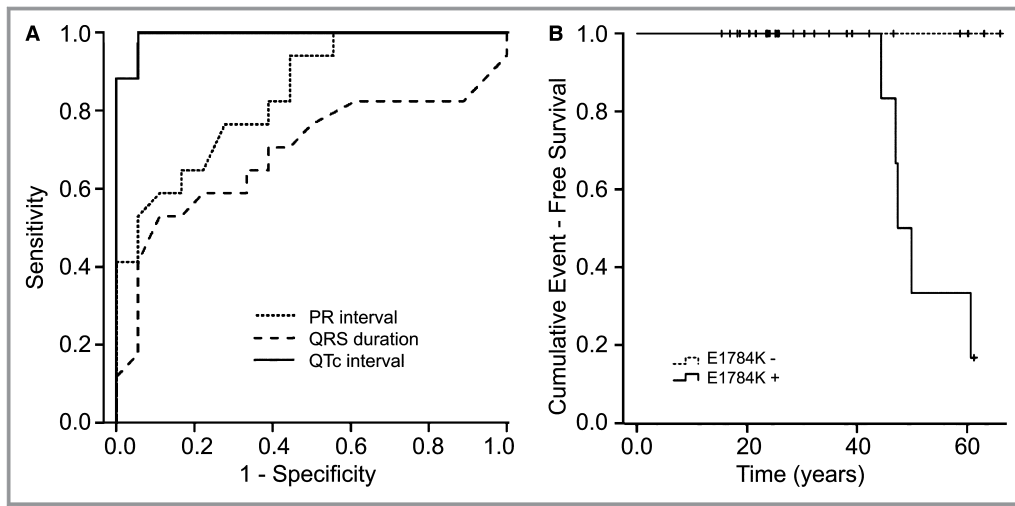
Figure 2. A, Receiver operating characteristic curve for PR interval, QRS duration, and QTc interval for the identification of *SCN5A*-E1784K carriers vs noncarriers. The best correlation was found for QTc interval (area under the curve 0.993). B, Kaplan–Meier event-free survival curves for *SCN5A*-E1784K mutation carrier vs noncarriers are displayed. Noncarriers had no cardiac events. Four mutation carriers developed sudden cardiac arrest and ventricular fibrillation beyond the age of 40 years.

Treatment and follow-up

The index patient (III,21) and his brother (III,18) received an ICD for secondary prophylaxis after successful resuscitation. The asymptomatic sisters of the proband (III,20 and III,22) with the electrocardiographic phenotype of LQTS and BrS were implanted with an ICD for primary prophylaxis after diagnosis due to the strong family history of SCD. All patients with a positive genotype were advised to avoid medication and circumstances, including fever, that might aggravate the phenotype and increase the risk for cardiac events according to www.crediblemeds.org and www.brugadadrugs.org.[16]

A total of 14 patients (82%) positive for *SCN5A*-E1784K mutation and the clinical phenotype of LQTS underwent ICD implantation. Three patients refused prophylactic ICD implantation. Including the index patient, 4 family members had VF and SCA and received adequate ICD shocks (2 males; mean

Table 2. Demographic and Electrocardiographic Parameters with Respect to Mutation and Polymorphism

	E1784K$^+$ n=17			E1784K$^-$ n=18		
	H558R$^+$ n=9	H558R$^-$ n=8	*P* Value	H558R$^+$ n=9	H558R$^-$ n=9	*P* Value
Male sex (%)	5 (56%)	3 (38%)	0.46	7 (78%)	4 (44%)	0.15
Age, y	35.1±16.3	25.7±13.9	0.22	17.6±9.4	40.4±14.6	0.002
RR, ms	911±215	930±315	0.88	822±196	909±132	0.29
PR, ms	208±34	183±22	0.94	154±15	170±16	0.04
QRS, ms	102±24	98±16	0.78	80±8	96±12	0.005
QTc, ms	464±17	470±15	0.42	412±15	404±20	0.34
QT dispersion, ms	44±20	36±24	0.74	29±14	28±16	0.95
QTp-e dispersion, ms	43±24	51±25	0.52	38±18	34±15	0.44
ST elevation in V1, mV	0.05±0.08	0.04±0.07	0.74	0.11±0.25	0.11±0.33	1.0
ST elevation in V2, mV	0.03±0.05	0.06±0.09	0.46	0	0.02±0.03	0.35
ST elevation in V3, mV	0.01±0.03	0.03±0.06	0.42	0.01±0.02	0.02±0.04	0.33
Brugada type II/III	5 (55%)	2 (25%)	0.2	0	0	1.0
Ajmaline positive	6/8 (75%)	7/7 (100%)	0.2	0/7	0/4	1.0

**Table 3.** Demographic and Electrocardiographic Parameters with Respect to Mutation and Age

	E1784K⁺ n=17			E1784K⁻ n=18		
	<40 n=11	≥40 n=6	*P* Value	<40 n=14	≥40 n=4	*P* Value
Male sex	5 (45%)	3 (50%)	1.0	9 (64%)	2 (50%)	1.0
Age, y	20.4±7.3	49.3±4.1	0.0001	21.7±10.0	55.1±3.6	0.001
RR, ms	804±219	1132±182	0.007	837±164	960±166	0.19
PR, ms	176±12	231±19	0.0001	166±10	181±12	0.02
QRS, ms	91±14	117±21	0.03	84±11	94±7	0.1
QTc, ms	465±15	470±19	0.55	408±18	407±17	0.96
QT dispersion, ms	36±21	48±22	0.29	25±11	39±15	0.13
QT$_{p-e}$ dispersion, ms	45±26	50±21	0.70	32±14	39±16	0.32
Max. ST, mV	0.03±0.06	0.13±0.08	0.02	0.01±0.03	0.02±0.05	0.63
Brugada type II/III	0	5 (83%)	0.001	0	0	1.0
Ajmaline positive	7/9 (78%)	6/6 (100%)	0.2	0/9	0/2	1.0

age 47.3±3 years) after a mean follow-up of 8±3 months (Figure 4). Clinical characteristics of patients with adequate ICD therapies or SCA during follow-up are presented in Table 5. One male patient (III,16), who refused an ICD implantation, also developed VF and SCA after 6.7 years of follow-up. All 5 patients were ≥40 years of age, had positive

Table 4. Electrocardiographic and Demographic Parameters with Respect to Genotype and Age

	E1784K⁺						E1784K⁻					
	H558R⁺			H558R⁻			H558R⁺			H558R⁻		
	<40 n=4	≥40 n=5	*P* Value	<40 n=7	≥40 n=1	*P* Value	<40 n=8	≥40 n=1	*P* Value	<40 n=5	≥40 n=4	*P* Value
Male sex	2	3	1.0	3	0	1.0	7	0	—	3	2	1.0
Age, y	18.5±5.6	48.3±3.7	0.0001	21.6±8.3	54.4	0.25	14.9±5.1	42.1	0.02	28.6±5.1	55.1±3.6	0.22
RR, ms	718±154	1065±89	0.02	854±246	1467	0.25	818±109	845	0.56	867±95	960±166	1.0
PR, ms	176±8	233±21	0.02	177±15	225	0.25	154±15	160	0.7	161±14.7	182±12	1.0
QRS, ms	89±17	111±25	0.286	98 ±16	106	05	79±7	73	1.0	92±12	101±13	0.22
QTc, ms	461±19	465±17	1.0	467±13	495	0.25	411±15	417	0.73	400±23	407±18	0.67
QT dispersion, ms	32±15	53±19	0.06	38±25	22	0.75	25±	41	0.11	19±10	39±15	0.22
QT$_{p-e}$ dispersion, ms	34±24	50±23	0.29	51±27	50	1.0	34±4	45	0.19	26±12	39±14	0.44
ST elevation in V1, mV	0	0.09±0.09	0.19	0.01±0.04	0.2	0.25	0.01±0.03	0	0.56	0	0.02±0.05	1.0
ST elevation in V2, mV	0	0.05±0.05	0.19	0.03±0.07	0.2	0.25	0	0	0.56	0	0.02±0.07	1.0
ST elevation in V3, mV	0	0.02±0.04	0.73	0.01±0.04	0.15	0.25	0	0	0.56	0	0.02±0.04	1.0
Brugada type II/III	0	4 (80%)	0.02	0	1	0.005	0	0	1.0	0	0	1.0
Ajmaline positive	1/3	5/5	0.17	6/6	1/1	1.0	0/6	0/1	1.0	0/2	0/2	1.0
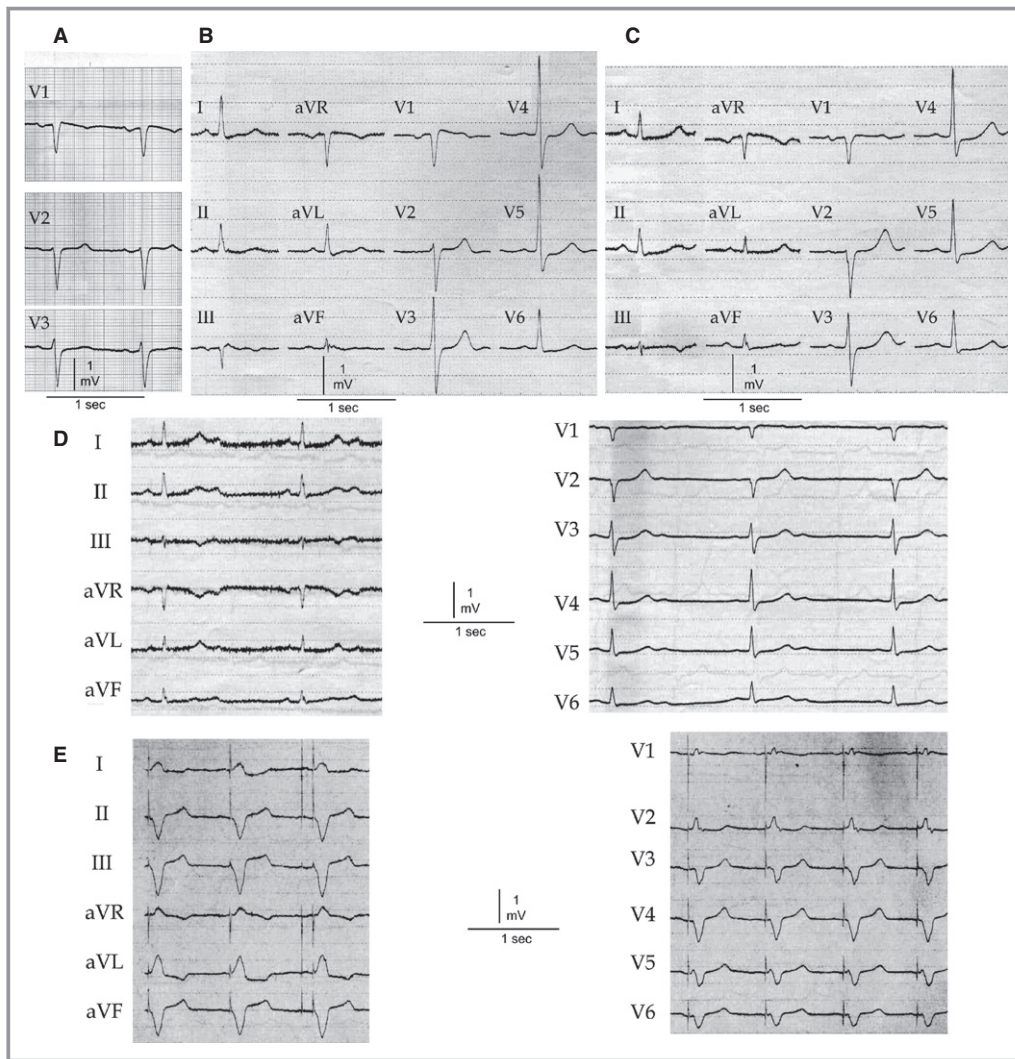
Figure 3. Basal ECGs of patient II,6. The ECGs show QTc prolongation, intermittent saddle-back type Brugada ECG in the right precordial leads, and progressive conduction disease. The patient developed second-degree atrioventricular block at the age of 78 years. A, Age 64: PR 193 ms, QRS 85 ms, QTc 461 ms. B, Age 75: PR 205 ms, QRS 94 ms, QTc 467 ms. C, Age 77: PR 229 ms, QRS 95 ms, QTc 472 ms. D, Age 78: Second-degree AV block, QRS 104 ms, QTc 465 ms. E, Age 81: DDD pacemaker ECG.

ajmaline challenge, first-degree AVB, and prolonged QTc interval. *SCN5A*-E1784K mutation and *SCN5A*-H558R polymorphism were found in each of them. Patients negative for *SCN5A*-E1784K mutations revealed no cardiac events (Figure 2B). A total of 6 stored intracardiac electrograms of adequate ICD therapies were analyzed in 4 cases. Patient III,18 had 2 episodes of VF. Mean basic cycle length before the inducing PVC was 770 and 576 ms. The VF-inducing PVC was coupled with 86% and 89% of the previous basic cycle length. Patients III,20 and III,22 had 1 and 2 adequate ICD shocks during follow-up, respectively. Basic cycle lengths were 888 ms in patient III,20 and 776 and 754 ms in patient III,22. Coupling interval of the VF-triggering PVC was 52% in these 2 cases. The proband (III,21) also developed VF during

follow-up. In his case, basic cycle length was 720 ms and the inducing PVC was short-coupled with 290 ms (40%).

Electrophysiology Study

Peak sodium current of *SCN5A*-E1784K

The *SCN5A*-E1784K mutation and the *SCN5A*-H558R polymorphism were reproduced in vitro on the *SCN5A* (hH1c) background in order to study the functional consequences of the mutation both with and without the presence of the polymorphism. We expressed WT+WT, E1784K+WT, and E1784K+H558R channels in TSA201 cells for whole-cell voltage-clamp measurements. Figure 5A represents a typical Na^+ current. Compared to the WT+WT group, the current
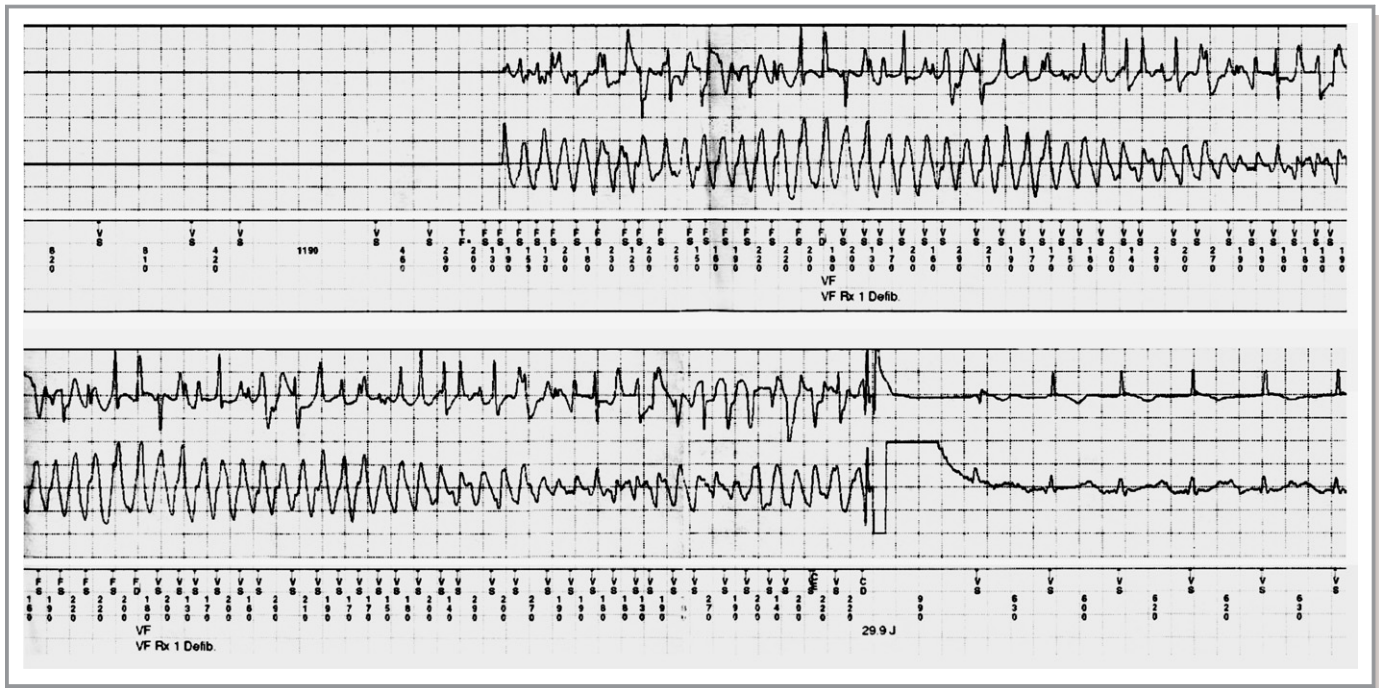
Figure 4. ECG from the ICD of patient III,22 positive for E1784K mutation and H558R polymorphism. Nine months after ICD implantation for primary prevention, the patient developed ventricular fibrillation, which was terminated by a single ICD shock. ICD indicates implantable cardioverter-defibrillator.

amplitude was moderately but significantly reduced for E1784K+WT. When H558R co-expressed with E1784K, the loss of function was slightly more prominent (Figure 5B). The maximum current amplitude was 665.14±87.60 pA/pF for WT+WT, 465.82±50.60 pA/pF for E1784K+WT, and 375.82±65.50 pA/pF for E1784K+H558R (Figure 5C). The voltage of peak sodium current was similar, and there was no difference of activation among the different groups (Figure 5D). Steady-state inactivation was shifted to much more negative potentials by the mutation, but were not further shifted in the presence of the H558R polymorphism (Figure 5E);

$V_{1/2}=-92.48\pm0.82$ mV and $k=5.98\pm0.30$ mV for WT+WT; $V_{1/2}=-103.51\pm1.34$ mV and $k=7.14\pm0.46$ mV for E1784K+WT; and $V_{1/2}=-106.54\pm1.78$ mV and $k=6.89\pm0.65$ mV for E1784K+H558R. $P\leq0.001$ is for difference in $V_{1/2}$ between WT and the other 2 groups. No significant difference in k was observed among all groups ($P=0.134$).

Late sodium current of SCN5A-E1784K

E1784K and H558R channels were also evaluated for late I_{Na} (Figure 6A). TTX-sensitive currents were compared at the end of a 300-ms depolarization, and values were obtained by

Table 5. Clinical and Demographic Data of Patients with Sudden Cardiac Arrest or Ventricular Fibrillation during Follow-Up

Patient	Sex	Event	Age With Event (y)	E1784K	H558R	PR	QRS	QTc	Ajmaline Test	ICD Implantation	CI (ms)	CI/Basic CL (%)
III,16	Male	SCA, VF	60.8	+	+	248	132	487	Positive	Refused	N/A	N/A
III,18	Male	SCA, VF	47.1	+	+	256	136	468	Positive	Yes	663	86.1%
											515	89.4%
III,20	Female	SCA, VF	50	+	+	238	105	456	Positive	Yes	460	51.8%
III,21 (proband)	Male	SCA, VF	47.5	+	+	205	108	465	Positive	Yes	290	40.3%
III,22	Female	SCA, VF	44.5	+	+	218	107	469	Positive	Yes	410	52.8%
											390	51.7%

CI indicates coupling interval of the first beat of polymorphic VT; CL, cycle length; ICD, implantable cardioverter-defibrillator; N/A, not available; SCA, sudden cardiac arrest; VF, ventricular fibrillation.
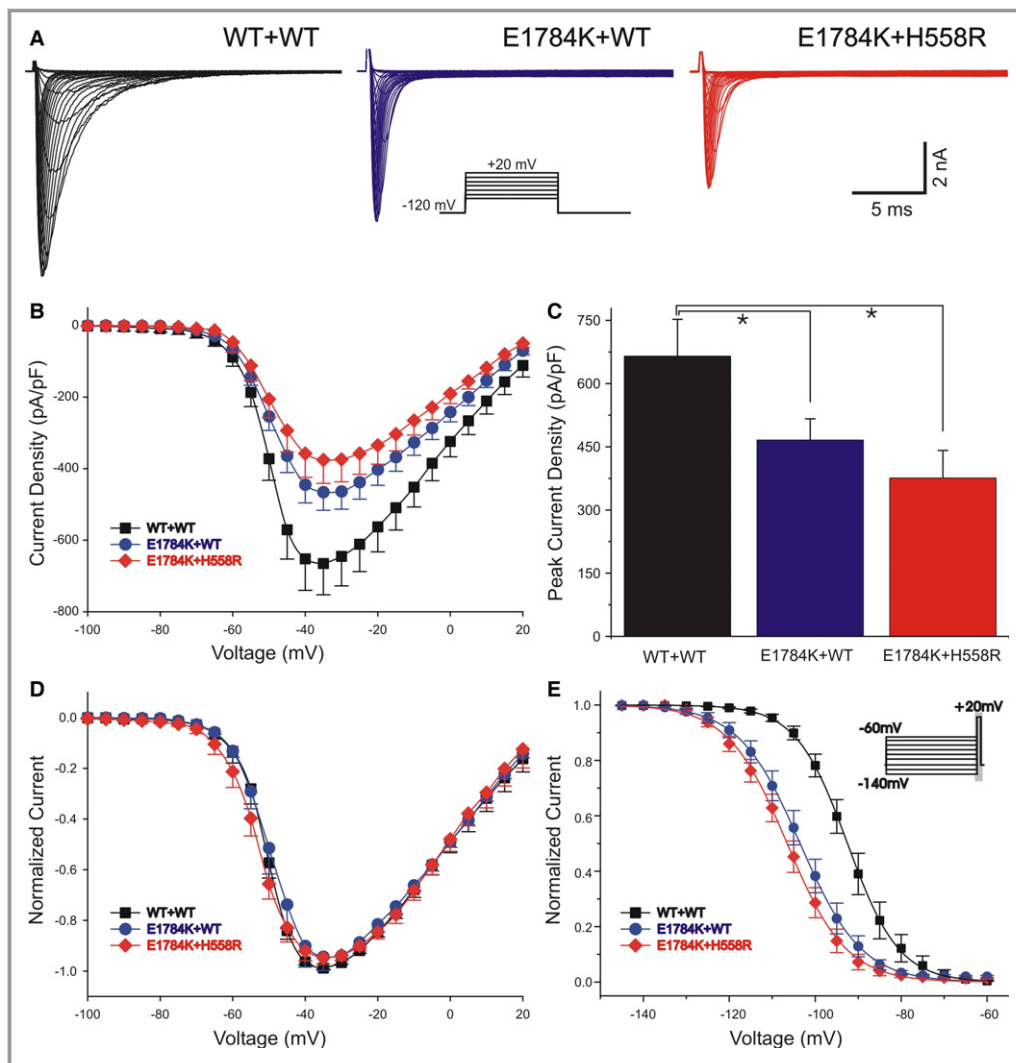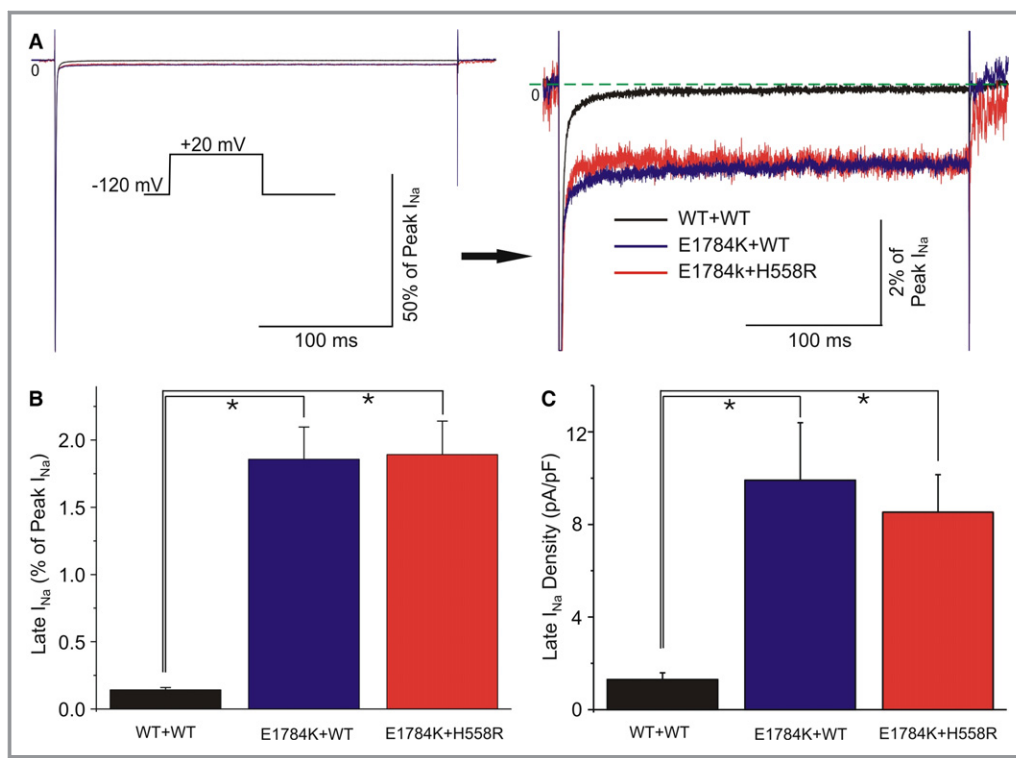
Figure 5. Functional expression studies evaluating the characteristics of I_{Na} for WT+WT, E1784K+WT, and E1784K+H558R. A, Whole-cell current traces from representative experiments of 3 groups. The voltage-clamp protocol is shown as the inset. I_{Na} was elicited by depolarizing pulses ranging from −90 mV to +20 mV in 5-mV increments with a holding potential of −120 mV. B, Current–voltage relationship for WT+WT (squares, n=33), E1784K+WT (circles, n=18), and E1784K+H558R (diamonds, n=11). C, Summary of maximum peak I_{Na} density of the 3 groups (*$P<0.05$). Peak E1784K+WT and E1784K+H558R current at −35 mV was smaller by 29.97% and 43.50% relative to WT+WT, respectively ($P=0.043$ and $P=0.032$). D, Normalized peak current–voltage relationships of the 3 groups. E, Steady-state inactivation for WT+WT (squares, n=22), E1784K+WT (circles, n=7), and E1784K+H558R (diamonds, n=12) using the standard protocol indicated in the diagram. Peak current was normalized to their respective maximum values and plotted against the conditioning potential. Data were fitted by Boltzman function. For values see text.

subtracting the values before and after TTX. Summary data for late I_{Na}, presented as percent of peak I_{Na}, were significantly higher when E1784K was expressed (1.86±0.23% co-expressed with WT, n=7; 1.89±0.25% co-expressed with H558R, n=10), compared with the WT group (0.14±0.02%, n=22; Figure 6B). Moreover, late I_{Na} amplitude recorded from the E1784K channel, with WT or H558R, was also obviously larger than in WT. Late I_{Na} density was 1.30±0.29 pA/pF (n=22) for WT+WT channels, 9.91±2.48 pA/pF (n=7) for E1784+WT and 8.53±1.63 pA/pF (n=10) for E1784+H558R

mutant channels (Figure 6C). H558R variant did not significantly alter increased late I_{Na} produced by *SCN5A*-E1784K mutation.

Discussion

SCN5A-E1784K is a frequently reported mutation in patients with LQT3 syndrome. It was first described as a congenital LQTS mutation caused by a defect in sodium channel inactivation giving rise to a small, persistent current.[6] These

Figure 6. Functional expression studies evaluating the characteristics of late I_{Na} for WT+WT, E1784K+WT, and E1784K+H558R. A, Representative TTX-sensitive sodium current traces obtained by subtraction, recorded during a 300-ms depolarization to −20 mV from −120 mV (left panel). Amplified traces showing late I_{Na} (right panel). B and C, Bar graph of relative late I_{Na} (% of peak I_{Na}) and late I_{Na} density (pA/pF) among 3 groups. Statistically significant differences (*P<0.05) were observed between E1784K+WT/E1784K+H558R and WT+WT in both panels (n=7, 10, 22 for each group). No statistically significant difference was observed between E1784K+WT and E1784K+H558R. For values see text.

characteristics were confirmed by several subsequent studies.[17,18] In 2000, Priori and co-workers reported that flecainide not only shortens the QT interval, but can also induce a BrS phenotype in *SCN5A*-E1784K carriers.[19] Two years later, it was confirmed by the same group that *SCN5A*-E1784K is associated with BrS in a study of 200 subjects diagnosed with BrS.[20] Recently, it has been reported as a mutation causing phenotypic overlap.[5,21] Of 41 *SCN5A*-E1784K carriers, 93% had LQT3, 22% had BrS, and 39% had SND.[5]

Here, we report on the clinical and electrocardiographic phenotypes of a large white family carrying the *SCN5A*-E1784K mutation and examine the modulatory effect of the common polymorphism H558R. Carriers of *SCN5A*-E1784K showed a strong genotype–phenotype correlation in this family, with a penetrance of 100% with respect to a prolonged QT interval. Additionally, 13 of 15 patients (87%) presented with the Brugada phenotype and 7% with SND. We demonstrate, for the first time, an association of *SCN5A*-E1784K with progressive CCD in mutation carriers above the age of 40 years. These electrocardiographic features are consistent with our finding of a loss of function of $I_{Na,P}$ and a gain of function of $I_{Na,L}$.

Genotype–Phenotype Correlation of *SCN5A*-E1784K

Compared to previous studies, patients positive for *SCN5A*-E1784K were found to exhibit the clinical phenotype of LQT 3 syndrome.[5,6] In the present cohort, the QTc cut-off of 445 ms was able to differentiate carriers from noncarriers with a sensitivity of 88% and a corresponding specificity of 100%. These findings are in line with in vitro studies showing a gain of function of late I_{Na}. In the present study, *SCN5A*-E1784K was associated with a 7.6-fold higher late I_{Na} density of E1784+WT compared with WT+WT channels. These electrophysiologic findings are comparable to those of previous studies.[5,6,17]

Priori *et al* reported overlapping clinical phenotypes of LQT3 syndrome and BrS. Thirteen patients with *SCN5A* mutations and LQTS received intravenous flecainide.[19] Three of 13 patients were carriers of *SCN5A*-E1784K. Following flecainide administration, 6 of 13 patients developed a

Brugada phenotype; 1 of the 3 patients (33%) was an *SCN5A*-E1784K carrier. In a larger study focusing on *SCN5A*-E1784K, Makita et al described an overlap of LQT3 and BrS in 9 of 41 mutation carriers.[5] Only 1 *SCN5A*-E1784K carrier showed spontaneous Brugada type 1 ECG. In the present study, the overlap of LQT3 and Brugada phenotype was much greater. At baseline, 7/17 *SCN5A*-E1784K carriers revealed a saddle-back–type ECG pattern. Following intravenous ajmaline, a diagnostic Brugada type-I ECG could be unmasked in 13/15 mutation carriers, whereas none of the *SCN5A*-E1784K-negative family members displayed a Brugada pattern. In our study the overlap of LQT3 and Brugada phenotype was significantly higher than in the study by Makita et al (87% *versus* 22%, respectively). The main difference between these 2 studies was the sodium channel blocker used. In previous studies, flecainide and pilsicainide were used to unmask the Brugada ECG pattern. In the present study, ajmaline was used. We have previously shown that ajmaline is significantly more sensitive than flecainide in provoking the Brugada phenotype. This may explain the higher overlap of Brugada and LQT3 phenotypes in the present study.[22]

Late sodium channel blockers, including mexiletine, flecainide, ranolazine, and GS-6615 have been used in patients with LQT3 for primary and secondary prevention. These agents reduced QTc and are expected to lower the arrhythmogenic risk.[23–25] Due to the high proportion of Brugada phenotypes in *SCN5A*-E1784K carriers, the Class IC sodium channel blockers, such as flecainide, should be avoided.[19] Whether ranolazine or GS-6615 are safe with respect to provocation of a Brugada phenotype in *SCN5A*-E1784K carriers is currently under investigation.[26,27]

SND has previously been associated with *SCN5A* mutations.[28] Makita et al recently reported that 16 of 41 *SCN5A*-E1784K carriers presented with SND.[5] In our family, only 1 patient (III,18; male; 47 years old) presented with SND characterized by persistent sinus bradycardia and chronotropic incompetence. This patient also carried *SCN5A*-H588R as well. His mother (II,6), an obligate carrier of *SCN5A*-E1784K and *SCN5A*-H558R, developed SND with increasing age. The mechanism underlying the *SCN5A*-E1784K-mediated sick sinus syndrome is likely due to the negative shift of steady-state inactivation (Figure 5) and the prolonged action potential duration caused by the increase in $I_{Na,L}$ (Figure 6).

Clinical manifestation of progressive cardiac conduction disease characterized by reduced excitability and conduction slowing of the His-Purkinje system results in prolongation of AV-conduction and widening of the QRS. *SCN5A* mutations have been shown to cause progressive cardiac conduction disease.[29,30] Our study is the first to associate *SCN5A*-E1784K with progressive cardiac conduction disease. *SCN5A*-E1784K carriers presented with slowed AV-nodal conduction and intraventricular conduction compared to noncarriers (PR:

196±31 ms *versus* 162±17 ms, $P<0.0001$; QRS: 100±20 ms *versus* 87±13 ms, $P=0.03$). Prolongation of AV and intraventricular conduction was age dependent. *SCN5A*-E1784K carriers below the age of 40 (mean age 20±7 years) displayed significantly shorter RR, PR, and QRS intervals than mutation carriers (average age, 49±4 years, Table 3). QTc interval was not significantly different between young and old mutation carriers. In the present large family, cardiac events occurred mainly in adulthood, above the age of 40. Thus, the addition of progressive CCD to prolonged QTc intervals and the Brugada phenotype may be responsible for the increased risk of SCD in this family.

Modulating Polymorphism—*SCN5A*-H558R

Single nucleotide polymorphisms have been shown to modify *SCN5A* mutations by either aggravation of the channelopathy or rescue of the pathophysiologic effect of the mutation. H558R is one of the most common *SCN5A*-polymorphisms, with an allelic frequency of 20% to 30% within individuals of white ethnicity.[7] In the general population, the presence of *SCN5A*-H558R (without any *SCN5A* mutation) has been reported to be associated with increased heart rate, longer QTc intervals, and an increased susceptibility to atrial fibrillation in population studies.[31–35] In the absence of sodium channel mutations, there is no functional effect of H588R detectable in heterologous expression systems in vitro.[35,36]

In patients with *SCN5A* mutations, the presence of H558R had diverse effects on the cellular and clinical phenotype. H558R has been shown to restore defective sodium channel function and/or trafficking of specific mutations (ie, R282H, T512I, D1275N, D1690N, G1748D, M1766L, V1951L, and P2006A) by modifying $I_{Na,P}$ and/or $I_{Na,L}$.[8,10,37–40] It is reported to improve ECG characteristics (QRS duration, J-point elevation, and "aVR sign") and clinical phenotype of BrS among carriers of *SCN5A* mutations.[12] In contrast, the sodium channel defects of other mutants (ie, E161K, R222Q, G400A, A572D, P1298L, R1632H, and I1835T) are aggravated in the *SCN5A*-H558R background.[13,36,38,41] In the case of other *SCN5A* mutations, including the missense and deletion mutants (ie, L212P, T220I, F1617Del, T187I, R878C, and G1408R), as well as the truncated mutants (ie, W1421X, K1578fs/52, R1623X), sodium currents are not significantly affected by H558R.[38]

In the present study, we evaluated the potential effect of *SCN5A*-H558R both in vitro and in vivo. When comparing electrocardiographic parameters of *SCN5A*-E1784K carriers, the presence or absence of *SCN5A*-H558R had no significant effect on PR, QRS, and QTc or the expression of a Brugada phenotype. In the in vitro study, co-expression of *SCN5A*-E1784K with H558R produced no significant effect on peak or late I_{Na}, although there was a tendency to a further reduction of $I_{Na,P}$. Above all, this kind of discrepancy of in vitro and

in vivo study may result from several possibilities. First, there are some other underlying mechanisms or modulating background among carriers, which are not present in the cell system used for experimental study. If we had been aware of those factors, we could conduct further investigation. Secondly, the intermittent severe phenotype, such as VT/VF or SCA, likely to be induced or triggered by certain acute conditions (ischemia, stress, fever, hypoxia, change of pH, etc), will not be duplicated in the normal in vitro study. Lastly, despite the chance being very small, there is still the possibility of coincidence. One of the evidences to support this assumption is that patients with SCA or VF during follow-up are the oldest carriers of *SCN5A*-E1784K, so the effect of aging on the mutant carriers could not be totally excluded.

Aging and Sodium Channel

There is some experimental evidence showing that sodium channels may decline with age. Huang et al demonstrated interesting results indicating an age-dependent alteration in expression and function of sodium current in rat heart.[42] Atrial fibrillation, a main arrhythmia significantly contributing to morbidity and mortality in elderly patients, has been correlated with enhanced age-dependent atrial fibrosis. Henry and his group showed decreased Nav1.5 expression and slowed conduction velocity by reduction of sodium channel expression and augmentation of fibrosis among an aged rat atrial fibrillation model. The effect was reversed by relaxin treatment in all aged animals with atrial fibrillation.[43] Although the role of aging on genetic variants in *SCN5A* remains unclear, it is reasonable to speculate that decreased sodium channel and increased fibrosis caused by aging will lead to a more serious phenotype for *SCN5A* mutation, such as what we observed in our *SCN5A*-E1784K family.

Risk of SCD

All 5 patients with SCA carried both the *SCN5A*-E1784K mutation and H558R polymorphism, and their phenotype presented as a combination of long QT interval, CCD, and type I Brugada ECG following ajmaline challenge, suggesting that *SCN5A*-H558R aggravated the *SCN5A*-E1784K⁻-associated phenotype under certain conditions. Interestingly, the mechanism of induction of ventricular tachyarrhythmias was different in the 4 patients, whose intracardiac electrograms were available. In 1 male patient, the coupling interval that triggered VF was 90% of the mean previous cycle length. This late coupled PVC suggests that prolonged QT interval is the underlying mechanism for SCA. However, in 2 female patients and the proband the coupling interval was short (40–52%), which indicates BrS as the responsible pathology for sudden death. From these findings, we cannot conclude which

phenotype is the responsible one for SCA in these patients. Possibly, both mechanisms are responsible within the same patient; thus, circumstances and drugs provoking either phenotype in these patients should be avoided.

Conclusions

We demonstrate high penetrance for LQTS, BrS, and CCD in a large family harboring the *SCN5A*-E1784K mutation. Prolonged QT interval was present at all ages, whereas CCD developed after the fourth decade of life. Moreover, at least 87% of *SCN5A*-E1784K carriers displayed ST-segment elevation diagnostic of BrS. This reveals one of the highest penetrances in a family with BrS syndrome reported by far. SCD/SCA cases occurred mainly after the fourth decade of life in carriers of both E1784K and H558R, displaying overlapping phenotypes of LQTS, BrS, and CCD. *In vivo* and *in vitro* studies showed no statistically significant differences in the electrocardiographic phenotypes or I_{Na} characteristics between E1784K with and without H588R.

Author Contributions

Hu, Veltmann, and Antzelevitch designed the study. Veltmann, Hu, Borggrefe, Wolpert, Schimpf, coordinated and collected the clinical evaluations. Hu and Antzelevitch supervised and coordinated the genetic and electrophysiology study. Pfeiffer and Cáceres did the genetic screening. Burashnikov provided the plasmids. Hu and Barajas-Martinez performed the electrophysiology laboratory work. Hu and Barajas-Martinez organized and summarized the genetic database. Veltmann, Hu, and Barajas-Martinez analyzed the data. Veltmann, Hu, and Antzelevitch wrote the manuscript. All co-authors contributed to editing of the manuscript.

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All authors have no financial or other considerations to disclose. All authors take responsibility for all aspects of the

reliability and freedom from bias of the data presented and their discussed interpretation.

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