Sodium Channelopathy Underlying Familial Sick Sinus Syndrome with Early Onset and Predominantly Male Characteristics

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Abe, Early-onset and male-predominant familial SSS

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ABSTRACT

Background – Sick sinus syndrome (SSS) is a common arrhythmia often associated with aging or organic heart diseases, but may also occur in a familial form with a variable mode of inheritance. Despite the identification of causative genes, including cardiac sodium channel (*SCN5A*), the etiology and molecular epidemiology of familial SSS remain undetermined primarily because of its rarity.

Methods and Results – We genetically screened 48 members of 15 SSS families for mutations in several candidate genes, and determined the functional properties of mutant sodium (Na) channels using whole-cell patch clamping. We identified six *SCN5A* mutations including a compound heterozygous mutation. Heterologously expressed mutant Na channels showed loss-of-function properties of reduced or no Na current density in conjunction with gating modulations. Among 19 family members with *SCN5A* mutations, QT prolongation and Brugada syndrome were associated in four and two individuals, respectively. Age of onset in probands carrying *SCN5A* mutations was significantly younger (12.4 ± 4.6 years, n=5, mean±SE) than that of *SCN5A*-negative probands (47.0 ± 4.6 years, n=10; p<0.001) or non-familial SSS (74.3 ± 0.4 years, n=538; p<0.001). Meta-analysis of SSS probands carrying *SCN5A* mutations (n=29) indicated profound male predominance (79.3%) resembling Brugada syndrome but with a considerably earlier age of onset (20.9 ± 3.4 years).

Conclusions –The notable pathophysiological overlap between familial SSS and Na channelopathy indicates that familial SSS with *SCN5A* mutations may represent a subset of cardiac Na channelopathy with strong male predominance and early clinical manifestations.

Keywords: sick sinus syndrome, *SCN5A*, mutation, gender

Introduction

Sick sinus syndrome (SSS), or sinus node dysfunction (SND), is a common clinical disorder that was first described in 1967,[1, 2] and is characterized by pathological sinus bradycardia, sinus arrest, chronotropic incompetence, and susceptibility to atrial tachycardia, especially atrial fibrillation. The syndrome comprises a variety of electrophysiological abnormalities in sinus node impulse formation and propagation, and represents the most frequent indication of pacemaker implantation.[3] SSS may be associated with underlying structural heart diseases, but most commonly occurs in the elderly in the absence of apparent accompanying heart disease. In three independent major trials of pacing in symptomatic SSS, the median or mean age was shown to be 73–76 years with both sexes affected approximately equally.[4-6] Although less common, SSS also occurs in young adults and children.

Recent studies including our own have linked several genetic defects with familial SSS, both with and without other concomitant cardiac conditions, mainly through candidate gene approaches. Implicated genes include the pore-forming α-subunit of the cardiac Na^+ channel (*SCN5A*),[7-11] hyperpolarization-activated cyclic nucleotide gated channel generating pacemaker current (*HCN4*),[12] and membrane adaptor protein ankyrin-B (*ANK2*).[13] Most recently, a genome-wide association study identified a rare missense variant of *MYH6*, the gene encoding the α-myosin heavy chain, which predisposes affected individuals to SSS.[14] Additionally, several other ion-channels and gap-junctions have been implicated in the SSS phenotype by knockout mice studies.[15] The majority of familial SSS cases exhibit autosomal dominant inheritance (OMIM_163800),[8-12] but an autosomal recessive disorder of compound heterozygous *SCN5A* mutations (OMIM_608567) also exists.[7, 16] Probands carrying compound heterozygous mutations typically manifest with severe clinical phenotypes including electrocardiogram (ECG) abnormalities with very early onset mostly during the first decade of life,[7] and often require the implantation of a pacemaker during infancy. Because familial SSS is relatively rare, the prevalence and functional consequences of these mutations and the epidemiologic characteristics have not been extensively studied.

In the present study, we investigated the clinical and genetic backgrounds of 15 families with SSS. We found that familial SSS with *SCN5A* mutations may represent a subset of cardiac Na channelopathy with strong male predominance and early clinical manifestations.

Methods

Clinical studies

The study population included 48 individuals from 15 unrelated Japanese families diagnosed with SSS. Family members underwent a physical examination, ECG, an exercise stress test, and Holter recording. SSS or SND was considered if one of the following conditions was recorded at one or more occasions when inappropriate for the circumstances: (a) sinus bradycardia; (b) sinus arrest or exit block; and (c) combinations of sinoatrial and atrioventricular (AV) conduction disturbances in conjunction with paroxysmal atrial tachyarrhythmias.[17] Long QT syndrome (LQTS) and Brugada syndrome (BrS) associated with SSS were diagnosed using the most recently available respective criteria.[18,19] Epidemiologic data of non-familial SSS (*n*=538) were obtained from the most recent databases of four Japanese institutions, in which SSS cases with a family history of pacemaker implantation, sudden death, or underlying structural heart diseases were excluded. This study was approved by a review committee of each institution and the subjects gave informed consent.

Genetic screening

All probands and family members who participated in the study gave their written informed consent in accordance with the Declaration of Helsinki and local ethics committees. Genetic analysis was performed on genomic DNA extracted from peripheral white blood cells using standard methods. Coding regions of *SCN5A*, *HCN4*, *KCNQ1*, *KCNH2*, *GJA5*, *KCNJ3*, *MYH6*, *IRX3*, and *LMNA* were amplified by PCR using exon-flanking intronic primers. Primer information for *KCNJ3*, *MYH6*,

IRX3, and *LMNA* is available online in Supplemental Table S1. Direct DNA sequencing was performed using an ABI 3130 genetic analyzer (Life Technologies, Carlsbad, CA). Mutations were validated by screening DNA samples from 200 healthy Japanese volunteers, and using public databases (dbSNP and 1000 Genomes).

Biophysical analysis of *SCN5A* mutants

Site-directed mutagenesis was performed using human heart Na channel α subunit Nav1.5. The human cell line tsA-201 was transiently transfected with wild-type (WT) or mutant *SCN5A* plasmids, and Na currents were recorded using the whole-cell patch clamp technique as described previously.[20] Further details are available in the online supplemental material.

Statistics

Results are presented as means ± SE, and statistical comparisons were made using the Student's *t*-test to evaluate the significance of differences between means followed by a Bonferroni adjustment for the total number of comparisons. Statistical significance was assumed for $P<0.05$.

Results

Case Presentations (Figures 1 and 2)

We genetically screened 48 members of 15 families with SSS (A1–A5 and B1–B10) and identified six *SCN5A* mutations in five families (A1–A5). The clinical and genetic information of 15 probands and mutation-positive family members ($n=14$) is shown in Supplemental Table S2.

Family A1: A 4-year-old boy (III:2) visited a pediatric clinic to investigate the bradycardia identified during a physical checkup at kindergarten. He had no perinatal problems. Despite a prescription of

denopamine, he experienced multiple syncopal episodes and visited a cardiology hospital at the age of 5 years. Holter ECG revealed SSS with a maximum RR interval of 5.9 s (Figure 2A), so an epicardial pacemaker was implanted. P wave amplitudes progressively diminished and had disappeared by the age of 12 when the pacemaker generator was replaced. However, atrial pacing could not be achieved even with the use of high voltages up to 6 V, compatible with atrial standstill. Genetic screening revealed two novel *SCN5A* mutations: an in-frame indel mutation 801_803delMSN/insS (c.2401_2409delinsTCC) in exon 15, referred to as MSN/S, and a missense mutation M1880V (c.5638A>G) in exon 28 (Figure 3). Heterozygous MSN/S was also demonstrated in paternal family members (II:1 and III:1) while heterozygous M1880V was observed in maternal family members (I:2, II:2, and III:3), demonstrating that the proband is a compound carrier of two distinct *SCN5A* mutations (Figure 1). There was no family history of SSS or pacemaker implantation, but his mother (II:2) was diagnosed with BrS from the observation of typical type-I ST elevation provoked by the sodium channel blocker flecainide (Figure 2B). The remaining affected members are asymptomatic and have no sign of cardiovascular diseases.

Family A2: An 18-year-old woman (II:2) was admitted to hospital because of dizziness upon standing. Holter ECG recording revealed frequent episodes of sinus arrest with a maximum RR interval of 7.7 s, so a diagnosis of SSS was made and a pacemaker was implanted. Echocardiography was normal. Her maternal uncle (II:3) died suddenly during running at the age of 35. Genetic screening revealed a missense mutation R219H (c.656G>A) in *SCN5A* exon 6 of the proband and her asymptomatic mother (II:2) (Figure 3). This mutation was previously reported in an individual with familial dilated cardiomyopathy (DCM) associated with third degree AV block, ventricular tachycardia, and atrial flutter (AFL).[21]

Family A3: A 3-year-old boy (II:2), admitted to hospital because of fever, showed AFL and

ventricular tachycardia (Figure 2C). Sinus arrest of 5.2 s was evident by Holter ECG recording, and he was diagnosed with SSS. Structural heart diseases were excluded by echocardiography. His mother also showed SSS and AFL. Genetic screening showed that they shared a novel heterozygous 2-bp deletion (c.5355_5354delCT) resulting in a frame shift mutation, L1786fsX2, located in exon 28 of *SCN5A* (Figure 3).

Family A4: A 15-year-old boy (III:5) with bradycardia lost consciousness after a collision during a soccer game. ECG displayed junctional bradycardia (heart rate, 38 bpm, max RR=5 s) with left axis deviation. Echocardiography revealed dilatation of the left ventricle (left ventricular end diastolic diameter, 59 mm) but the left ventricular (LV) systolic function was normal (ejection fraction, 64%). His brother (III:1), a pacemaker recipient because of SSS, has a dilated right ventricle and has experienced episodes of AFL and ventricular tachycardia. Genetic screening revealed a missense mutation D1275N (c.3823G>A) in *SCN5A* exon 21, previously linked to DCM with conduction disorder (Figure 3).[22] The mutation was identified in the brother (III:1) as well as in his asymptomatic father (II:1) and younger sister (III:7).

Family A5: Sinus bradycardia and QT prolongation with day-to-day variation (QTc: 450–530 ms) were observed when the proband (II:2) was 22 years old, which were exacerbated after thyroidectomy as a treatment of hyperthyroidism at the age of 36. An electrophysiological study revealed SND (sinus node recovery time, 5.08 s), AV block (His ventricular, 68 ms), and atrial standstill in addition to QT prolongation (QTc=522 ms, Figure 2D), whereas the thyroid function was normally controlled. Her mother (I:2) and son (III:3) showed QT prolongation, while her brother (II:3) had both LQTS and BrS. Genetic screening revealed that the proband and these three family members carried an *SCN5A* missense mutation in exon 28, E1784K (c.5350C>A) (Figure 3), which is the most common *SCN5A* mutation in LQT3 associated with multiple clinical phenotypes of LQTS,

BrS, and SSS.[20] The proband prophylactically received an implantable cardioverter defibrillator at the age of 36, which discharged appropriately 1 year later during an episode of spontaneous ventricular fibrillation.

Mutation analysis of probands and family members

Nineteen mutation carriers were identified within the five families with SSS (A1–A5) (Supplemental Table S2). Seven individuals (37%) exhibited SSS, while seven carriers (37%) were asymptomatic; other carriers showed variable arrhythmias including LQTS, BrS, and AFL without SSS, suggesting that SSS has a considerably reduced penetrance in these families. No mutations were identified in *HCN4*, *KCNQ1*, *KCNH2*, *KCNJ3*, *MYH6*, *GJA5*, and *IRX3*. Among the *SCN5A* mutations we found, D1275N[22, 23] and E1784K[20] have previously been well-characterized; therefore, we analyzed the functional properties of other mutants.

Functional characterization of *SCN5A* mutations

As shown in Figure 4A, all plasmids, except for L1786fsX2, elicited a robust Na current but the non-inactivating late current, which characterizes type-3 LQTS mutations,[24] was not evident. Peak current density measured 24 h after transfection was significantly reduced in MSN/S, M1880V+MSN/S, R219H, and L1786fsX2 compared with WT (Figure 4B, 4C). Because L1786fsX2 was non-functional, channel properties were further analyzed for M1880V+MSN/S and R219H (biophysical properties of other mutations are shown in Supplemental Table S3). The voltage-dependence of activation was significantly shifted in the depolarizing direction (+7.5 mV, $p<0.01$) in M1880V+MSN/S, and the voltage-dependence of steady-state inactivation was significantly shifted in the hyperpolarizing direction in R219H (−11.4 mV, $p<0.01$) (Figure 4D). Recovery from inactivation was remarkably delayed in R219H (Figure 4E). The lower current density, depolarizing shift of the activation curve, hyperpolarizing shift of the inactivation curve, and

delayed recovery from inactivation observed in M1880V+MNS/S, R219H, and the non-functional channel L1786fsX2 are typical loss-of-function properties of Na channels.

Epidemiological and genetic characteristics of familial SSS

The average age of onset of the probands in our SSS cohort was 35.5 ± 5.4 years (range, 3–65), which was substantially younger than the 538 cases of sporadic SSS (74.3 ± 0.4 years, $p<0.001$, Figure 5). When the cohort was classified by the presence or absence of *SCN5A* mutations, the *SCN5A*-positive subgroup showed an even earlier onset (12.4 ± 4.6 years, $n=5$) than the negative subgroup (47.0 ± 4.6 years, $n=10$; $p<0.001$). To confirm this observation, we searched the literature for descriptions of SSS probands with *SCN5A* mutations and a family history of SSS, and identified 24 cases in addition to the five (A1–A5) in our cohort (Supplemental Table S4). To our surprise, the 29 SSS probands with *SCN5A* mutations not only exhibited an early onset (20.9 ± 3.4 years) but also a striking male predominance (male: 23/29, 79.3%). If we focus on the SSS subgroup without disease complications such as BrS or LQTS, the tendency of early onset was even more obvious. As shown in the histogram in Figure 5B, the SSS-only subgroup (filled boxes) had a very young age of onset (mean age, 7.8 ± 1.9 years, $n=11$) and a prominent male preponderance (10/11; 91%). These data indicate that the subset of familial SSS with *SCN5A* mutations has a strong male predominance resembling BrS, but exhibits a considerably earlier clinical manifestation. Nevertheless, the same pathophysiological basis of loss-of-function of the cardiac Na channel is shared.

Discussion

We identified six *SCN5A* mutations in 15 familial SSS probands, and demonstrate that familial SSS with *SCN5A* mutations may represent a distinct cardiac Na channelopathy with early onset and male predominance.

SCN5A is the cardiac Na channel gene responsible for the generation and rapid propagation of

action potentials in the heart. Mutations in *SCN5A* have been linked to a wide range of inherited lethal arrhythmias, referred to as “cardiac sodium channelopathy”, including type-3 LQTS (LQT3),[24] BrS,[25] progressive cardiac conduction defect,[26] sudden infant death syndrome, and SSS. To date, at least 27 distinct *SCN5A* mutations have been reported that are causative of SSS, although some mutation carriers exhibit mixed clinical phenotypes in addition to SSS (Supplemental Table S4).[8-10] Heterologously expressed mutant *SCN5A* commonly results in a loss-of function with reduced (R219H, M1880V+MSN/S) or no (L1786fsX2) Na current density, in conjunction with alterations of biophysical properties (Figure 3).

The compound heterozygous mutation M1880V+MSN/S results in a channel behavior phenotype that is intermediate between that of M1880V and MSN/S, and functional analysis of the singular mutations suggests that M1880V may have more benign channel properties than MSN/S (Supplemental Table S3). However, the proband’s mother (A1-II:2), carrying the M1880V allele but not MSN/N, showed BrS, suggesting that the *in vivo* consequences of M1880V may not be as benign as observed in the heterologous expression system. A similar discrepancy between *in vivo* and *in vitro* situations was previously reported in the *SCN5A* mutation D1275N, which was identified in the A4 family of our present cohort. Despite the severe conduction disturbance and DCM, the heterologously expressed D1275N channel showed almost normal behavior.[27] Interestingly, cardiomyocytes from mice carrying the human D1275N *SCN5A* allele display both a decreased current density and late Na current, which is a hallmark of LQT3.[23] Such a mixed biophysical phenotype is observed in association with several *SCN5A* mutations including E1784K (identified in family A5), the most frequent *SCN5A* mutation causative of LQTS, BrS, and SSS.[20] Moreover, a negative shift of steady-state inactivation is a common biophysical mechanism underlying the phenotypic overlap of cardiac Na channelopathy.[20] Taken together, the most typical biophysical feature in *SCN5A* mutations causative of SSS is the loss-of-function property that reduces electrical coupling between the sinus node and surrounding atria resulting in conduction block (exit block).

However, a subset of *SCN5A* mutations associated with SSS display a gain-of-function property characteristic of LQT3,[9, 20] resulting in a reduction of the sinus rate by prolonging the sinus node action potential and disrupting its complete repolarization.[9]

Recently, Gousselin-Badaroudine *et al.* identified the R219H mutation in a family with DCM associated with ventricular tachycardia and third degree AV block.[21] They found that the mutant R219H channel selectively permeates protons through the channel pore, which in turn results in severe LV dysfunction and conduction disturbance. By contrast, clinical observations of our R219H carriers (A2-I:2, II:2) were rather benign, with electrical abnormalities restricted to the sinus node with no LV dysfunction. Moreover, we observed a reduced peak Na current, a hyperpolarizing shift of steady-state inactivation, and a slowed recovery from inactivation of the R219H mutant channel. However, we were unable to evaluate the proton permeation properties in our experimental system. Such loss-of-function properties are commonly observed in most *SCN5A* mutations responsible for familial SSS. The reasons for the discrepancy between our findings and those of Gousselin–Badaroudine *et al.* are not clear; however, additional genetic modifiers within *SCN5A* or other unidentified genes may contribute to the severe clinical and/or biophysical properties of mutant Na channels.

Autosomal dominant transmission is the most common mode of inheritance in familial SSS,[8, 10, 11] although autosomal recessive transmission has been reported in several severe juvenile cases of congenital SSS.[7, 16] Consistent with previous reports,[4-6] the majority of patients with non-familial SSS in the present study are elderly, with both genders nearly equally affected (Figure 5). By contrast, our familial SSS cases associated with *SCN5A* mutations are characterized by early onset and a strong male predominance. Male predominance (80–90%) and prevalence of *SCN5A* mutations (≈20%) are known features of BrS,[19] which often associates with SND or atrial arrhythmias.[28, 29] Makiyama *et al.* genetically screened 38 BrS probands and identified four *SCN5A* mutation carriers (10.5%), all of which were complicated with bradyarrhythmias including SSS.[11] These data suggest a

close relationship between BrS and familial SSS, and our study further supports this notion by demonstrating the prominent male predominance in these two disorders.

Nonetheless, there is a clear difference between familial SSS and BrS regarding the age of manifestation. The mean age of the 29 probands of familial SSS with *SCN5A* mutations in our study were considerably younger (20.9 ± 3.4 years) than those affected with BrS, which typically manifests during adulthood with a mean age of around 40 years. Furthermore, it should be noted that only two of 24 (8.3%) of the family members of our cohort that were carriers of the mutations exhibited a BrS phenotype even later in their lives (mean age: 34.5 ± 4.1 years), suggesting that penetrance of familial SSS in our cohort was incomplete (67%; 16/24). These data suggest that SND is the earliest electrophysiological manifestation of *SCN5A* mutation carriers, which may be associated with other arrhythmias such as LQT3, BrS, or DCM under the control of confounding factors including aging, hormones, other genetic variations, and undetermined environmental factors. We have followed-up the SSS probands for 7.7±2.1 years (Supplemental Table S2), but longer-term follow-up of the mutation carriers as well as further genetic studies of mutation-negative SSS probands may uncover crucial factors that determine the distinct age-dependent manifestations observed in familial SSS and BrS.

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Disclosures

None

References

1. Lown B. Electrical reversion of cardiac arrhythmias. *Br Heart J*. 1967;29:469-489.
2. Ferrer MI. The sick sinus syndrome in atrial disease. *JAMA*. 1968;206:645-646.
3. Mangrum JM, DiMarco JP. The evaluation and management of bradycardia. *N Engl J Med*. 2000;342:703-709.
4. Connolly SJ, Kerr CR, Gent M, Roberts RS, Yusuf S, Gillis AM, Sami MH, Talajic M, Tang AS, Klein GJ, Lau C, Newman DM. Effects of physiologic pacing versus ventricular pacing on the risk of stroke and death due to cardiovascular causes. Canadian Trial of Physiologic Pacing Investigators. *N Engl J Med*. 2000;342:1385-1391.
5. Andersen HR, Thuesen L, Bagger JP, Vesterlund T, Thomsen PE. Prospective randomised trial of atrial versus ventricular pacing in sick-sinus syndrome. *Lancet*. 1994;344:1523-1528.
6. Lamas GA, Lee KL, Sweeney MO, Silverman R, Leon A, Yee R, Marinchak RA, Flaker G, Schron E, Orav EJ, Hellkamp AS, Greer S, McAnulty J, Ellenbogen K, Ehlert F, Freedman RA, Estes NA, 3rd, Greenspon A, Goldman L, Mode Selection Trial in Sinus-Node Dysfunction. Ventricular pacing or dual-chamber pacing for sinus-node dysfunction. *N Engl J Med*. 2002;346:1854-1862.
7. Benson DW, Wang DW, Dyment M, Knilans TK, Fish FA, Strieper MJ, Rhodes TH, George AL, Jr. Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (SCN5A). *J Clin Invest*. 2003;112:1019-1028.
8. Smits JP, Koopmann TT, Wilders R, Veldkamp MW, Opthof T, Bhuiyan ZA, Mannens MM, Balser JR, Tan HL, Bezzina CR, Wilde AA. A mutation in the human cardiac sodium channel (E161K) contributes to sick sinus syndrome, conduction disease and Brugada syndrome in

two families. *J Mol Cell Cardiol*. 2005;38:969-981.

9. Veldkamp MW, Wilders R, Baartscheer A, Zegers JG, Bezzina CR, Wilde AA. Contribution of sodium channel mutations to bradycardia and sinus node dysfunction in LQT3 families. *Circ Res*. 2003;92:976-983.

10. Makita N, Sasaki K, Groenewegen WA, Yokota T, Yokoshiki H, Murakami T, Tsutsui H. Congenital atrial standstill associated with coinheritance of a novel SCN5A mutation and connexin 40 polymorphisms. *Heart Rhythm*. 2005;2:1128-1134.

11. Makiyama T, Akao M, Tsuji K, Doi T, Ohno S, Takenaka K, Kobori A, Ninomiya T, Yoshida H, Takano M, Makita N, Yanagisawa F, Higashi Y, Takeyama Y, Kita T, Horie M. High risk for bradyarrhythmic complications in patients with Brugada syndrome caused by *SCN5A* gene mutations. *J Am Coll Cardiol*. 2005;46:2100-2106.

12. Schulze-Bahr E, Neu A, Friederich P, Kaupp UB, Breithardt G, Pongs O, Isbrandt D. Pacemaker channel dysfunction in a patient with sinus node disease. *J Clin Invest*. 2003;111:1537-1545.

13. Mohler PJ, Splawski I, Napolitano C, Bottelli G, Sharpe L, Timothy K, Priori SG, Keating MT, Bennett V. A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. *Proc Natl Acad Sci U S A*. 2004;101:9137-9142.

14. Holm H, Gudbjartsson DF, Sulem P, Masson G, Helgadottir HT, Zanon C, Magnusson OT, Helgason A, Saemundsdottir J, Gylfason A, Stefansdottir H, Gretarsdottir S, Matthiasson SE, Thorgeirsson GM, Jonasdottir A, Sigurdsson A, Stefansson H, Werge T, Rafnar T, Kiemeney LA, Parvez B, Muhammad R, Roden DM, Darbar D, Thorleifsson G, Walters GB, Kong A, Thorsteinsdottir U, Arnar DO, Stefansson K. A rare variant in MYH6 is associated with high risk of sick sinus syndrome. *Nat Genet*. 2011;43:316-320.

15. Dobrzynski H, Boyett MR, Anderson RH. New insights into pacemaker activity: promoting understanding of sick sinus syndrome. *Circulation*. 2007;115:1921-1932.

16. Kodama T, Serio A, Disertori M, Bronzetti G, Diegoli M, Narula N, Grasso M, Mazzola S, Arbustini E. Autosomal recessive paediatric sick sinus syndrome associated with novel compound mutations in SCN5A. *Int J Cardiol*. 2013;167:3078-3080.

17. Olgin JE, Zipes DP. Specific arrhythmias: Diagnosis and treatment. In: Braunwald E, Zipes DP, Libby P, eds. *Heart disease: a textbook of cardiovascular medicine*. Philadelphia, PA: W.B. Saunders Company; 2001:815-899.

18. Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: from genetics to management. *Circ Arrhythm Electrophysiol*. 2012;5:868-877.

19. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, LeMarec H, Nademanee K, Perez Riera AR, Shimizu W, Schulze-Bahr E, Tan H, Wilde A. Brugada syndrome: Report of the second consensus conference: Endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation*. 2005;111:659-670.

20. Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L, Schulze-Bahr E, Fukuhara S, Mochizuki N, Makiyama T, Itoh H, Christiansen M, McKeown P, Miyamoto K, Kamakura S, Tsutsui H, Schwartz PJ, George AL, Jr., Roden DM. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. *J Clin Invest*. 2008;118:2219-2229.

21. Gosselin-Badaroudine P, Keller DI, Huang H, Pouliot V, Chatelier A, Osswald S, Brink M, Chahine M. A proton leak current through the cardiac sodium channel is linked to mixed arrhythmia and the dilated cardiomyopathy phenotype. *PLoS One*. 2012;7:e38331.

22. McNair WP, Ku L, Taylor MR, Fain PR, Dao D, Wolfel E, Mestroni L, Familial Cardiomyopathy Registry Research G. SCN5A mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. *Circulation*. 2004;110:2163-2167.

23. Watanabe H, Yang T, Stroud DM, Lowe JS, Harris L, Atack TC, Wang DW, Hipkens SB,

Leake B, Hall L, Kupershmidt S, Chopra N, Magnuson MA, Tanabe N, Knollmann BC, George AL, Jr., Roden DM. Striking In vivo phenotype of a disease-associated human SCN5A mutation producing minimal changes in vitro. *Circulation*. 2011;124:1001-1011.

24. Bennett PB, Yazawa K, Makita N, George AL, Jr. Molecular mechanism for an inherited cardiac arrhythmia. *Nature*. 1995;376:683-685.

25. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G, Ortiz-Lopez R, Wang Z, Antzelevitch C, O'Brien RE, Schulze-Bahr E, Keating MT, Towbin JA, Wang Q. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature*. 1998;392:293-296.

26. Schott JJ, Alshinawi C, Kyndt F, Probst V, Hoorntje TM, Hulsbeek M, Wilde AA, Escande D, Mannens MM, Le Marec H. Cardiac conduction defects associate with mutations in SCN5A. *Nat Genet*. 1999;23:20-21.

27. Groenewegen WA, Firouzi M, Bezzina CR, Vliex S, van Langen IM, Sandkuijl L, Smits JP, Hulsbeek M, Rook MB, Jongsma HJ, Wilde AA. A cardiac sodium channel mutation cosegregates with a rare connexin40 genotype in familial atrial standstill. *Circ Res*. 2003;92:14-22.

28. Bordachar P, Reuter S, Garrigue S, Cai X, Hocini M, Jais P, Haissaguerre M, Clementy J. Incidence, clinical implications and prognosis of atrial arrhythmias in Brugada syndrome. *Eur Heart J*. 2004;25:879-884.

29. Morita H, Fukushima-Kusano K, Nagase S, Miyaji K, Hiramatsu S, Banba K, Nishii N, Watanabe A, Kakishita M, Takenaka-Morita S, Nakamura K, Saito H, Emori T, Ohe T. Sinus node function in patients with Brugada-type ECG. *Circ J*. 2004;68:473-476.

Figure Legends

Figure 1. Familial SSS pedigrees with *SCN5A* mutations. Probands are arrowed. In family A1, the proband (III:2) had compound heterozygous mutations of MSN/S (p.801_803delMSN/ins) and M1880V, while I:2, II:1, and III:1 had MSN/S; I:4, II:2, and II:3 had M1880V. Of 19 mutation carriers, seven individuals were asymptomatic (penetrance=63%).

Figure 2. Electrocardiographic phenotypes. A, Consecutive strips of Holter ECG recording from proband A1-III:2 carrying compound heterozygous *SCN5A* mutations showed sinus arrest for 5.9 s (at the age of five). B, His mother A1-II:2 showed coved-type ST elevation in V1-V2 leads during the flecainide challenge test. C, Paroxysmal AFL recorded in the proband A3-II:2. D, QT prolongation (QTc=522 ms) remain evident in the proband A5-II:2 even after thyroid hormone supplemental therapy.

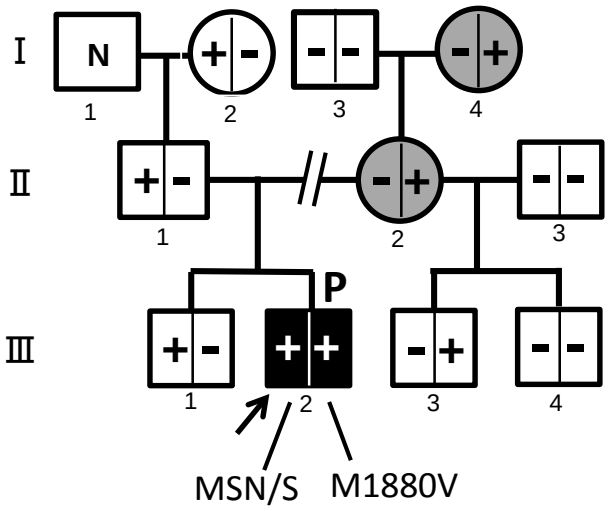
Figure 3. Sequencing of *SCN5A*. Schematic of the transmembrane topology of *SCN5A* representing the location and sequencing electropherogram of each mutation. Three mutations are located in the transmembrane domains, and three are in the cytoplasmic C-terminal.

Figure 4. Whole-cell current recordings of wild-type (WT) and mutant Na channels. A, Representative whole-cell current traces obtained from tsA-201 cells transfected with WT or mutant Na channels. Currents were recorded from a holding potential of −120 mV and stepped to various membrane potentials from −90 mV to +50 mV for 20 ms. B, Current was normalized to cell capacitance to give a measure of Na current density. There were significant decreases in maximum current density in M1880V+MSN/S and R219H ($p<0.05$), as well as in MSN/S and L1786fsX2 ($p<0.01$) compared with WT. C, Current-voltage relationship of WT and two mutant channels

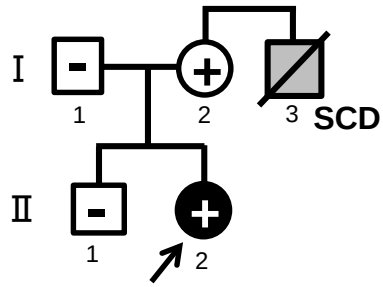
(MSN/S+M1880V and R219H). D, Steady-state inactivation and conduction-voltage relationship in MSN/S+M1880V and R219H. E, Time course of recovery from inactivation at −120 mV. Detailed parameters are provided in Supplemental Table S3.

Figure 5. Age of onset and gender difference in probands with non-familial and familial SSS. A, Age of onset of non-familial SSS (*n*=538), SSS probands of our cohort (*n*=15) including *SCN5A* mutation-negative (*n*=10) or positive (*n*=5), and meta-analysis of 29 cases with *SCN5A* mutations. § and * indicate $p<0.001$ vs. mutation-negative, and $p<0.001$ vs. non-familiar SSS, respectively. B, Upper histogram shows age of onset in Japanese patients with non-familial SSS (*n*=538, 74.3 ± 0.4 years); male (upper, *n*=241) and female (lower, *n*=257). There was no gender difference. Lower histogram shows the age of onset and gender difference in 29 probands with familial SSS. Filled and shaded columns show SSS-only (*n*=11) and SSS with complications including BrS (*n*=18), respectively. Early onset and male predominance were more apparent in the SSS-only group.

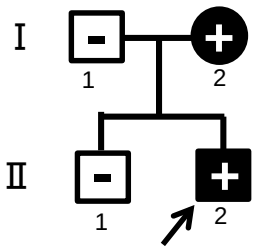
Family A1 (*SCN5A*: M1880V+MSN/S)



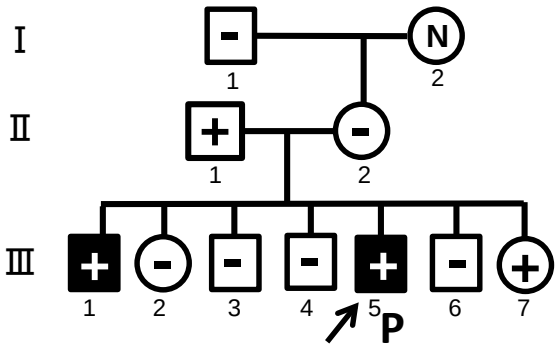
Family A2 (*SCN5A*: R219H)



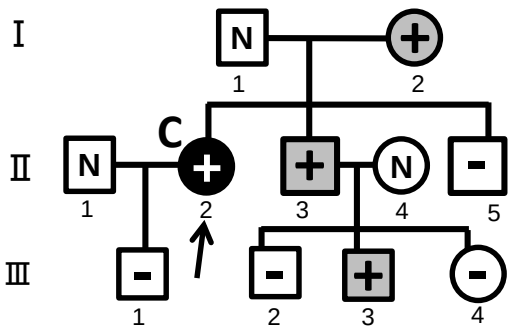
Family A3 (*SCN5A*: L1786fsX2)



Family A4 (*SCN5A*: D1275N)



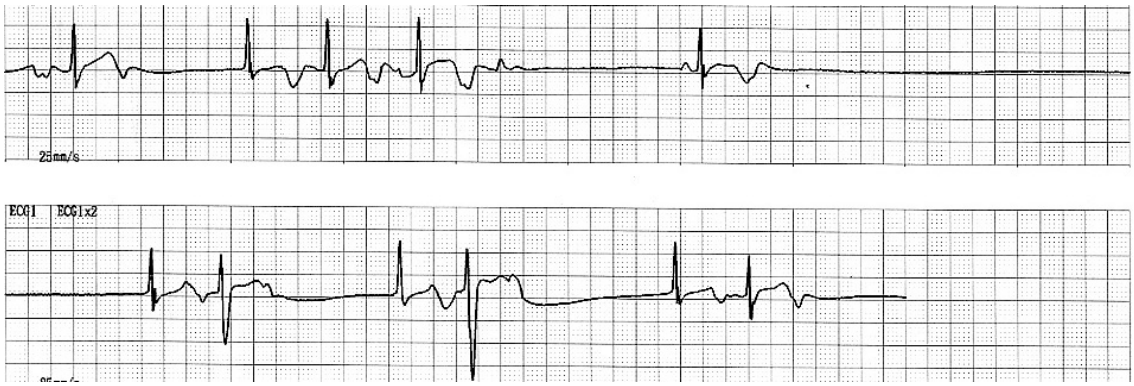
Family A5 (*SCN5A*: E1784K)



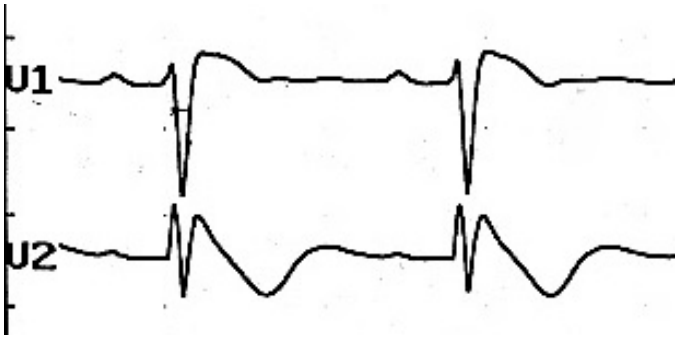
■	SSS
▩	Other arrhythmias
□	No arrhythmia
+	Mutation carrier
-	Non-carrier
SCD	Sudden Cardiac Death
N	No genetic testing
P	Pacemaker
C	ICD

Figure 2. Abe *et al*.

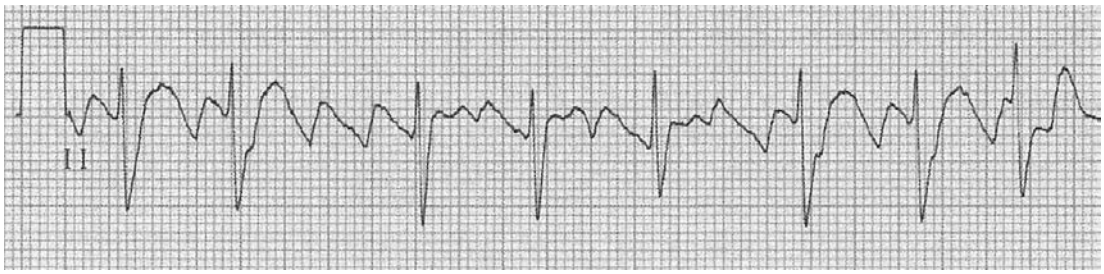
A



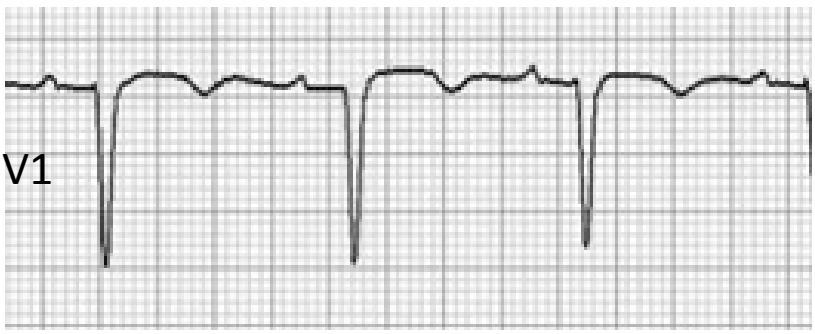
B

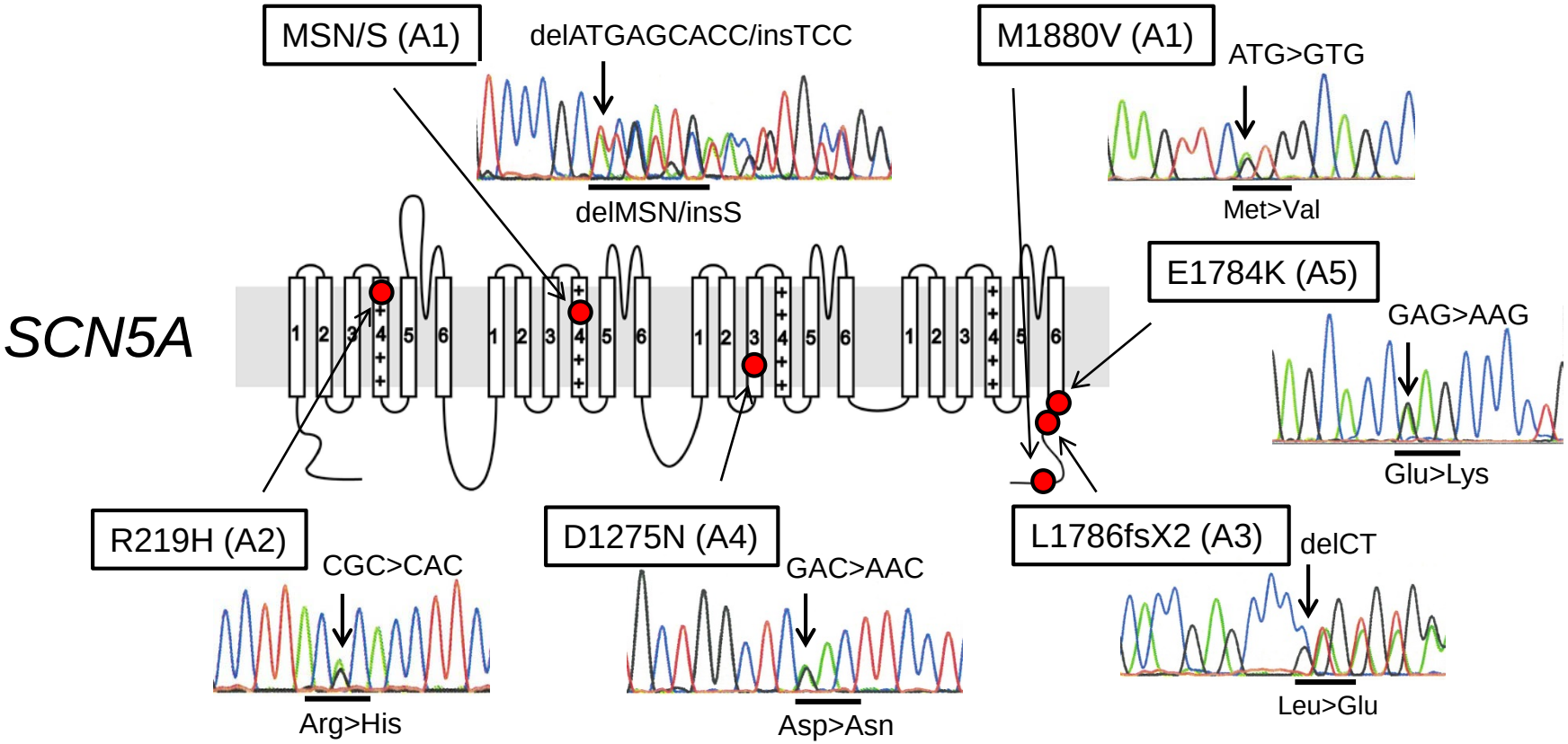


C



D




SCN5A
MSN/S (A1)
delATGAGCACC/insTCC
delMSN/insS
M1880V (A1)
ATG>GTG
Met>Val
E1784K (A5)
GAG>AAG
Glu>Lys
R219H (A2)
CGC>CAC
Arg>His
D1275N (A4)
GAC>AAC
Asp>Asn
L1786fsX2 (A3)
delCT
Leu>Glu

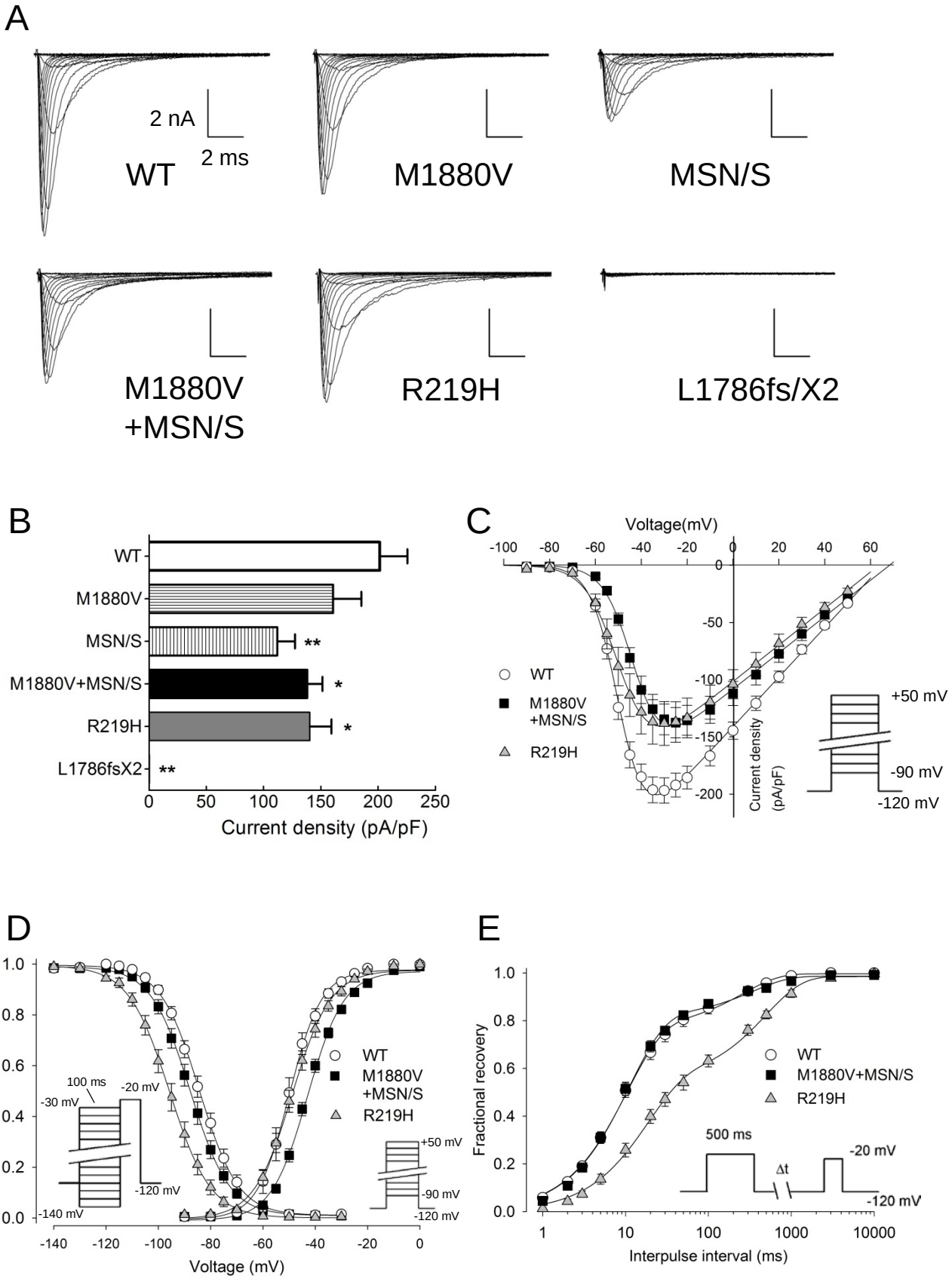
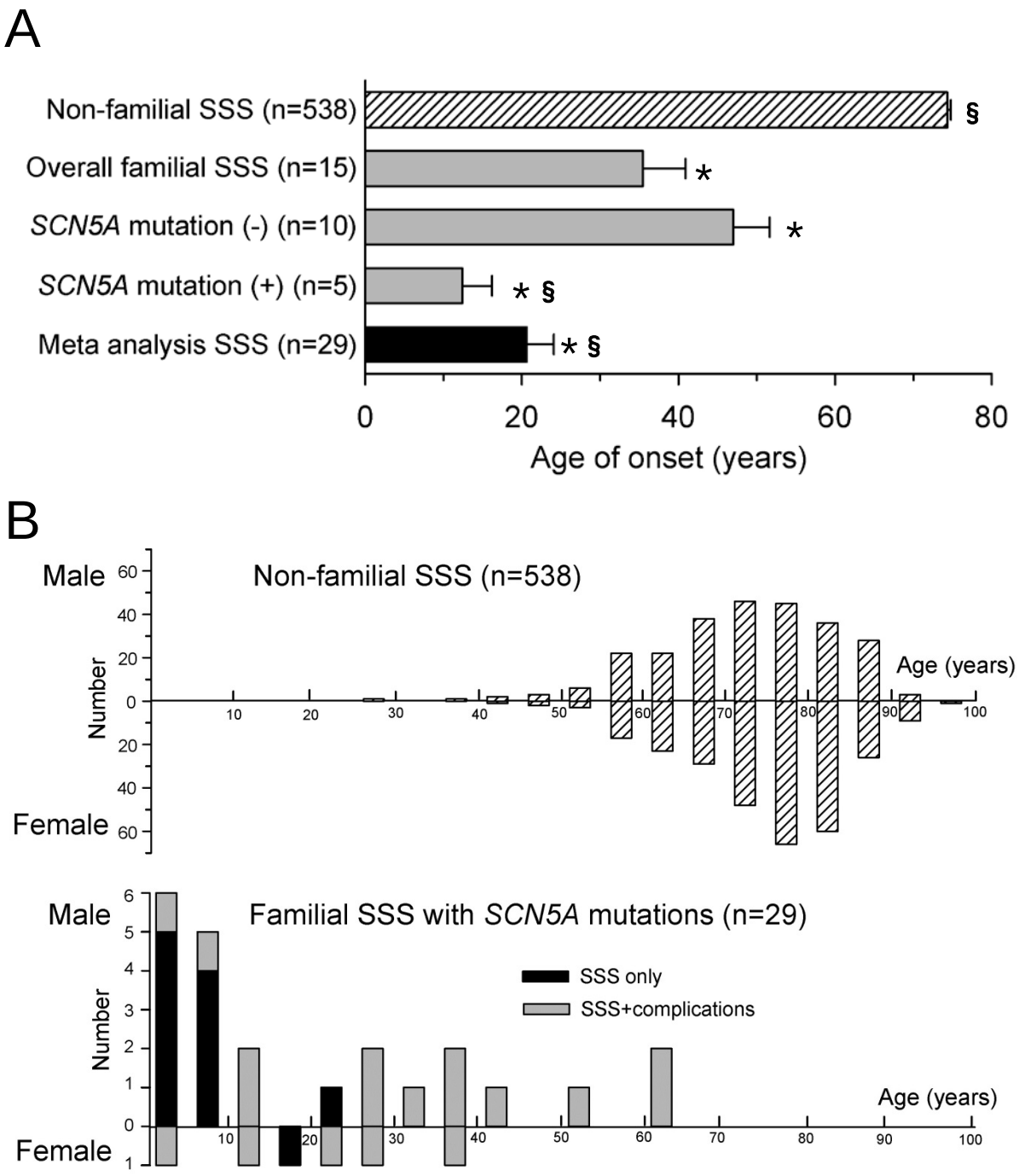

A
2 nA
2 ms
WT
M1880V
MSN/S
M1880V
+MSN/S
R219H
L1786fs/X2
B
WT
M1880V
MSN/S
M1880V+MSN/S
R219H
L1786fsX2
**
*
*
**
0
50
100
150
200
250
Current density (pA/pF)
C
Voltage(mV)
-100
-80
-60
-40
-20
0
20
40
60
0
-50
-100
-150
-200
Current density (pA/pF)
WT
M1880V
+MSN/S
R219H
+50 mV
-90 mV
-120 mV
D
Channe availability/
Normalized conductance
1.0
0.8
0.6
0.4
0.2
0.0
100 ms
-20 mV
-30 mV
-120 mV
-140 mV
WT
M1880V
+MSN/S
R219H
+50 mV
-90 mV
-120 mV
-140
-120
-100
-80
-60
-40
-20
0
Voltage (mV)
E
Fractional recovery
1.0
0.8
0.6
0.4
0.2
0.0
WT
M1880V+MSN/S
R219H
500 ms
Δt
-20 mV
-120 mV
1
10
100
1000
10000
Interpulse interval (ms)

Figure 5. Abe *et al.*



SUPPLEMENTAL MATERIAL

Table I. Nucleotide sequences of the primers used for the mutational analysis

Gene	Analyzed region	Forward primer (5' to 3')	Reverse primer (5' to 3')
KCNJ3	Ex1	TAAGCAGTCAGTTTGGGGGTAC	CGGGCTGGTTCTCAGACGAGT
	Ex2	TTCGGCCCATTTGCTAGAACATAGT	TAAACTCTGAGTCATTTCGCCCAAT
	Ex3	CTATTCTATTATTCGGGCTTC	CCCGGAACTGAACTTATTCGT
MYH6	Ex3	AGAGGACAAAGCCACTCGCTG	TGCAAGTGGCTCCACCTCTG
	Ex4-6	AATGGGAAGGGAAATTACCTG	CTAGGCATCAGCGTGTCTGC
	Ex7-8	CCCTGTATGGAGAACAGTAG	TGGGTGTGGCAAAACAGCAC
	Ex9-10	CATTTCCAGAACCATCCAGG	CCTGCATGCAGGAGTCGTTG
	Ex11-12	TTGCCTGGTGCAGACATGCTG	AGAGAGCCTGGTCAGCACCTC
	Ex13	GTGCTCACTTATCCTTTCCC	CTCTCAGCAAATGGCTGTTG
	Ex14	CAACAGCCATTTGCTGAGAG	CTCTAGTTTCTTGGGTGTAG
	Ex15	TGTCAGGGTATGGGACTGTG	GTGCTTTGAAGCAGCAGGAC
	Ex16-20	AAAGTCTCAGAGCTACCAAGCG	CTTCTGACCCACACTAGTTGAC
	Ex21-23	AGTCTACGTGCCTACGAACTTG	CAGGACTTTCTGGGCCATTGG
	Ex24-25	GAAGGAGGCAAAAGAGCATAC	CTGCAGCCTCAGTTACCTCAG
	Ex26-28	TTCCTGGTAGCTTTTCAGAGC	TCCATTTCTGGCACTGAGATG
	Ex29-30	AAGGCTGGGCTTGGTTGAAG	AGCCGCATGTCCAAGATCTG
	Ex31-32	CAGATCTTGGACATGCGGCT	AGATTTTGTCCTGGGGTCAG
	Ex33-34	ACCGTGTATCTTCTCATCCTC	ACTCAGTAGGTTTCCACAAGG
	Ex35-36	ACCACCTTTAATTCTTTCTGG	TAAATCTACCAACAGCATCTC
	Ex37	GGGAAAGGTGATTGCATTTGC	AGCAAACTCTTTGTCCAGGCC
	Ex38	GTTGCAGGAATATGCATGAGG	ACATATAGGGCAAGCAGTGCC
	Ex39	ACCACAAGTGCCTCTAACGTG	CTACTGCCCTGATCCAGGATG
IRX3	Ex1	CCCGTAGAAATGTCAATCAG	AACAAACCTCACAGCGAATG
	Ex2	TCCCGCAGCTGGTAAGAG	GCAGAAAGCAGGAGTGGAGA
	Ex3	GGGCGGGCTCTGCGGGTAGT	GCGTGGTGCTCGGCGTCCT
	Ex4-6	CCCACCCTACAGTTAAACC	TACACGGACATGCTTACAAG
LMNA	Ex1	GCGCACTCCGACTCCGAGCAG	TCCGGGGAGGGAGGAGACTAT
	Ex2	CCCTCTCCTGGTAATTGCAG	AAGATGTGACCTCAGTGGGTAAAA
	Ex3-5	GCCCTCCTTCCCTGGACCTGT	CTCTGTGGTTGTGGGGACACTT
	Ex6-7	CCAGTTGCCAGCCAAGACTATGTT	CTCTGTGGTTGTGGGGACACTT
	Ex8-10	GGGCCTTTGAGCAAGATACACC	GGCCAGGCCAGCGAGTAAAGTTC
	Ex11	CTGAGCCTTCTCCCCTTTTATGTC	CAGAGGTGGGCTGTCTAGGACTC
	Ex12	AGGGATGGGGGAGATGCTAC	CCCCTCCCATGACGTGCAGG

Table II. Clinical and genetic characteristics of 15 families of SSS

Individual	Age at Dx (proband)	Sex	Current age	Gene	Mutation	Phenotype	Device
A1-I:2		F	66	*SCN5A*	MSN/S	Asymptomatic	No
A1-I:4		F	67	*SCN5A*	M1880V	WPW syndrome	No
A1-II:1		M	41	*SCN5A*	MSN/S	Asymptomatic	No
A1-II:2		F	40	*SCN5A*	M1880V	BrS	No
A1-III:1		M	21	*SCN5A*	MSN/S	Asymptomatic	No
A1-III:2*	4	M	19	*SCN5A*	M1880V + MSN/S	SSS, AS	PPM
A1-III:3		M	13	*SCN5A*	M1880V	Asymptomatic	No
A2-I:2		F	53	*SCN5A*	R219H	Asymptomatic	No
A2-II:2*	18	F	26	*SCN5A*	R219H	SSS	No
A3-I:2		F	49	*SCN5A*	L1786fsX2	SSS	No
A3-II:2*	3	M	12	*SCN5A*	L1786fsX2	SSS, AFL, VT	No
A4-II:1		M	52	*SCN5A*	D1275N	Asymptomatic	No
A4-III:1		M	26	*SCN5A*	D1275N	SSS, AFL, VT	PPM
A4-III:5*	15	M	18	*SCN5A*	D1275N	SSS, DCM	PPM
A4-III:7		F	15	*SCN5A*	D1275N	Asymptomatic	No
A5-I:2		F	67	*SCN5A*	E1784K	LQTS	No
A5-II:2*	22	F	38	*SCN5A*	E1784K	SSS, LQTS, VF	ICD
A5-II:3		M	36	*SCN5A*	E1784K	BrS+LQTS	No
A5-III:3		M	11	*SCN5A*	E1784K	LQTS	No
B1*	62	F	70	NA		SSS	PPM
B2*	65	F	72			SSS, AVB	PPM
B3*	56	M	61			SSS, HCM	PPM
B4*	39	F	42			SSS	PPM
B5*	52	F	54			SSS, Epilepsy	CRT-P
B6*	17	M	19			SSS, AS, AVB, LVNC	PPM
B7*	52	M	53			SSS	No
B8*	49	F	51			SSS, AFL, VT	ICD
B9*	48	F	49			SSS, AFL, VT	ICD
B10*	30	F	62			SSS, AFL, VT	PPM→ICD

*: Probands, Dx: diagnosis, MSN/S: 801_803delMSN/insS, AFL: Atrial flutter, AS: Atrial standstill,
VT: Ventricular tachycardia, AVB: Atrioventricular block, BrS: Brugada syndrome, LQTS: Long QT syndrome
PPM: Permanent pacemaker, ICD: Implantable cardioverter defirillator
CRT-P: Cardiac resynchronization therapy pacemaker, NA: not available
HCM: Hypertophyc cardiomyopathy, DCM: dilated cardiomyopathy, LVNC: Left ventricular non-compaction
Follow-up period of the probands: 7.7±2.1 years (n=15)

Table III. Channel properties of novel *SCN5A* mutations identified in familial SSS

Parameters/ Channel (n)		WT (15)	M1880V (10)	MSN/S (13)	M1180V+MSN/S (14)	R219H (11)	L1786fsX2 (3)
Peak INa density (pA/pF)		201.4 ± 24.2	160.7 ± 24.8	111.9 ± 15.5**	138.2 ± 13.2*	140.3 ± 19.0*	0
Activation	$V_{1/2}$ (mV)	-50.5 ± 1.5	-42.7 ± 1.6**	-36.3 ± 0.7**	-43.0 ± 0.8**	-48.8 ± 1.3	NA
	k (mV)	-4.9 ± 0.3	-6.4 ± 0.3**	-7.2 ± 0.3**	-6.4 ± 0.3**	-6.9 ± 0.2**	
Steady-state inactivation	$V_{1/2}$ (mV)	-84.1 ± 1.3	-90.4 ± 1.5**	-80.7 ± 0.9	-87.8 ± 1.4	-95.5 ± 1.7**	NA
	k (mV)	7.1 ± 0.3	6.8 ± 0.1	7.0 ± 0.2	6.9 ± 0.1	7.2 ± 0.2	
Recovery from inactivation	τ_{fast} (ms)	10.6 ± 0.8	9.7 ± 1.0	20.6 ± 1.7**	11.9 ± 1.1	20.6 ± 1.7**	NA
	τ_{slow} (ms)	293 ± 27	217 ± 6	520 ± 78**	396 ± 43*	564 ± 76**	
	A_{fast}	0.75 ± 0.01	0.82 ± 0.00	0.81 ± 0.00	0.82 ± 0.00*	0.56 ± 0.00**	
	A_{slow}	0.23 ± 0.01	0.16 ± 0.00	0.19 ± 0.00	0.16 ± 0.00*	0.42 ± 0.00**	

*****:p<0.05, ******: p<0.01, NA: Not applicable

Table IV. Age of onset and the gender of 29 probands of familial SSS associated with *SCN5A* mutations

Familial SSS probands without complications

case	Mutation 1	Mutation 2	Age of onset (years)	Sex (male%)	Complications	Reference
1	T220I	R1623X	9	M	-	1
2	P1298L	G1408R	6	M	-	1
3	D349N	D1790N	2	M	-	2
4	delF1617	R1623H	5	M	-	1
5	L212P	-	3	M	-	3
6	R878C	-	8	M	-	4
7	D1275N	-	22	M	-	5
8	F1775fsX15	-	6	M	-	6
9	M1880V	MSN/S	4	M	-	This study (A1)
10	R219H	-	18	F	-	This study (A2)
11	L1786fsX2	-	3	M	-	This study (A3)
Total (n=11)			7.8±1.9	Male 10/11 (91%)		

Familial SSS probands with complications

case	Mutation 1	Mutation 2	Age of onset (years)	Sex	Complications	Reference
1	T187I	-	33	M	BrS	7
2	D356N	-	61	M	BrS	7
3	R367H	-	37	M	BrS	8
4	G514C	-	3	F	CCD	9
5	D1275N	-	29	M	DCM, CCD	10, 11
6	D1275N	-	29	F	DCM, CCD	12
7	K1578fsX52	-	52	M	BrS	7
8	D1596H	-	7	M	DCM	12
9	R1623X	-	61	M	BrS	7
10	V1763M	-	0	M	LQT	13
11	E1784K	-	36	M	LQT	14
12	E1784K	-	13	F	LQT	14
13	E1784K	-	13	M	LQT	14
14	E1784K	-	44	M	LQT	14
15	E1784K	-	39	F	LQT	14
16	1795isD	-	27	M	LQTS, BrS, CCD	15
17	D1275N	-	15	M	DCM	This study (A4)
18	E1784K	-	22	F	LQT	This study (A5)
Total (n=18)			28.7±4.6	Male 13/18 (72%)		

Overall Familial SSS probands with *SCN5A* mutations

Total (n=29)	20.9±3.4	Male 23/29 (79%)	