patients could have been misdiagnosed as having ERS under the previous criteria.

Significance of Brugada-pattern electrocardiogram in the right precordial leads in early repolarization syndrome

Higher intercostal recordings in leads V_1 and V_2 were reported to show similar prognostic value as standard recordings in BrS.[3,5,14] Even in this study, six patients with type 1 BrP-ECG and five patients with non-type 1 BrP-ECG only in the high costal spaces showed a similar recurrence rate of arrhythmia as BrS patients in previous studies who were diagnosed by the standard ECG.

Regarding the significance of non-type 1 BrP-ECG in inferolateral ERS, we previously reported that such ERS patients had clinical profiles similar to BrS patients with high recurrence rates of VF and electrical storm.[10] In this study, in which 30% of ERS patients had BrP-ECG only in the HICS, VF mostly recurred in patients showing BrP-ECG in any of the right precordial leads including HICS and these patients comprised 50% of our ERS cohort diagnosed under the previous criteria for ERS, although the remaining ERS patients without BrP-ECG exhibited a favorable outcome and clinical profiles dissimilar to BrS. Furthermore, the presence of BrP-ECG showed a good positive predictive value (63%) to identify VF recurrence during ~110 months of follow-up. This indicates that BrP-ECG can be a reliable marker of poor outcome in patients with ER or ERS. So far, useful predictors for sudden death due to VF have not been identified by both retrospective and prospective studies on ERS, although wider distribution of J-waves in inferolateral leads and horizontal/descending ST segment following J-waves were reported to be weak predictors for sudden death in patients with ER by retrospective studies in which the cause of sudden death was never identified.[18–20] On the other hand, the registry of BrS patients in Japan,[22] in which the prognosis of individuals with non-type 1 BrP-ECG was investigated prospectively in addition to the prognosis of those with type 1 ECG, contains records that 1 of 7 individuals with inferolateral J-waves and non-type 1 BrP-ECG on the standard ECG, who were not included in this study, developed VF during 40.7 ± 16.4 months of follow-up.

Early repolarization syndrome has been considered a clinical entity different from BrS, although they share a similar genetic background and represent a continuous spectrum of phenotypic expression.[20] Quinidine and isoproterenol were reported to be the first-line therapy for suppressing VF even in ERS patients, the exact reasons for which are unknown.[1,21] This study clarified that 12 of 14 ERS patients with VF recurrence had BrP-ECG in any of the right precordial leads. This means that many of the previously reported ERS patients with poor prognosis might have included patients with type 1 BrP-ECG only in the high costal ECG recordings or non-type 1 BrP-ECG in any lead, which could account for the clinical similarity of half of the ERS patients to BrS patients and the effectiveness of quinidine and isoproterenol in suppression of VF storm due to ERS. In contrast, most of the ERS patients without BrP-ECG in any of the right precordial leads exhibited a favorable outcome and clinical profiles dissimilar to BrS. We previously reported that ERS consisted of two heterogeneous subtypes with or without non-type 1 BrP-ECG.[10] Provided that half of the ERS cases are nearly identical to BrS, the remaining ERS cases without BrP-ECG may be considered as true ERS possibly caused by a different mechanism. A systematic search for BrP-ECG with high intercostal ECG recordings and drug challenge test is required not only to exclude BrS but also to classify the subgroups of ERS.

Study limitations

This study was conducted at a single center using retrospective analysis. The small number of patients might limit the interpretation of the results; nevertheless, it should be pointed out that the number of patients with ERS is comparable to that in previous multicenter studies. Further prospective multicenter studies with larger numbers of patients will be needed to confirm these results.

Conclusions

This study showed that 16% of the patients diagnosed with ERS under the previous criteria were actually BrS patients with inferolateral ER and a type 1 BrP-ECG only in HICS that would not have been previously recognized unless appropriate recordings were performed. Thirty-four percent of the ERS patients with non-type 1 BrP-ECG in any of the right precordial leads including HICS were at high risk for arrhythmic events, in contrast to the 50% of ERS patients without BrP-ECG who were at very low risk for recurrent events despite a previous VF episode. These results also indicated that the presence of BrP-ECG can be a reliable marker of poor outcome in patients with ER or ERS. High intercostal ECG recording may be considered essential not only to exclude BrS but also to stratify the risk of ERS.

Funding

This work was supported by a research grant for the cardiovascular diseases (grant number H26-040) from the Ministry of Health, Labor, and Welfare, Japan, and intramural research funds (22-1-2, M25-74) for cardiovascular disease from National Cerebral and Cardiovascular Center.

Conflict of interest: none declared.

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Circ Arrhythm Electrophysiol. 2014;7:511-517; originally published online April 24, 2014;
doi: 10.1161/CIRCEP.113.001340
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1941-3149. Online ISSN: 1941-3084

Original Article

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

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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 Na channel (*SCN5A*), the pathogenesis 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 Na channels using whole-cell patch clamping. We identified 6 *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 4 and 2 individuals, respectively. Age of onset in probands carrying *SCN5A* mutations was significantly less (mean±SE, 12.4±4.6 years; n=5) than in *SCN5A*-negative probands (47.0±4.6 years; n=10; $P<0.001$) or nonfamilial 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. ***(Circ Arrhythm Electrophysiol.* 2014;7:511-517.)**

Key Words: mutation ■ $Na_v1.5$ voltage-gated sodium channel ■ sex ■ sick sinus syndrome

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 3 independent major trials of pacing in symptomatic SSS, the median or mean age was shown to be 73 to 76 years with both sexes affected approximately equally.[4–6] Although less common, SSS also occurs in young adults and children.

Clinical Perspective on p 517

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]

Received December 5, 2013; accepted March 22, 2014.

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The Data Supplement is available at http://circep.ahajournals.org/lookup/suppl/doi:10.1161/CIRCEP.113.001340/-/DC1.

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Circ Arrhythm Electrophysiol **is available at http://circep.ahajournals.org** **DOI: 10.1161/CIRCEP.113.001340**

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 severe clinical phenotypes including ECG abnormalities with 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 epidemiological 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 ≥1 occasions when inappropriate for the circumstances: (1) sinus bradycardia, (2) sinus arrest or exit block, and (3) combinations of sinoatrial and atrioventricular 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] Epidemiological data of nonfamilial SSS (n=538) were obtained from the most recent databases of 4 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 polymerase chain reaction using exon-flanking intronic primers. Primer information for *KCNJ3*, *MYH6*, *IRX3*, and *LMNA* is available in Table I in the Data Supplement. 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 $Na_v1.5$. The human cell line tsA-201 was transiently transfected with wild-type 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 Methods in the Data Supplement.

Statistics

Results are presented as means±SE, and statistical comparisons were made using the Student *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

We genetically screened 48 members of 15 families with SSS (A1–A5 and B1–B10) and identified 6 *SCN5A* mutations in 5 families (A1–A5; Figures 1 and 2). The clinical and genetic information of 15 probands and mutation-positive family members (n=14) is shown in Table II in the Data Supplement.

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 R-R interval of 5.9 s (Figure 2A), so an epicardial pacemaker was

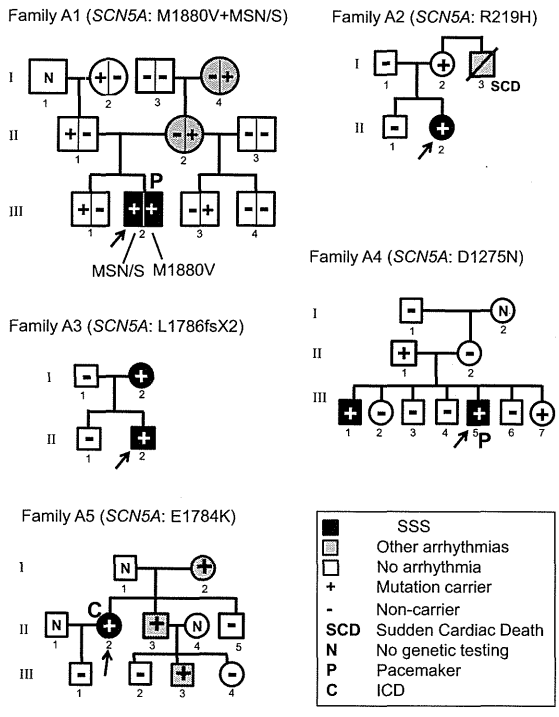

Figure 1. Familial sick sinus syndrome (SSS) pedigrees with *SCN5A* mutations. Probands are arrowed. In family A1, the proband (III:2) had compound heterozygous mutations of MSN/S (p.801_803delMSN/ins) and M1880V, while I:2, II:1, and III:1 had MSN/S; I:4, II:2, and II:3 had M1880V. Of 19 mutation carriers, 7 individuals were asymptomatic (penetrance, 63%). ICD indicates implantable cardioverter defibrillator.

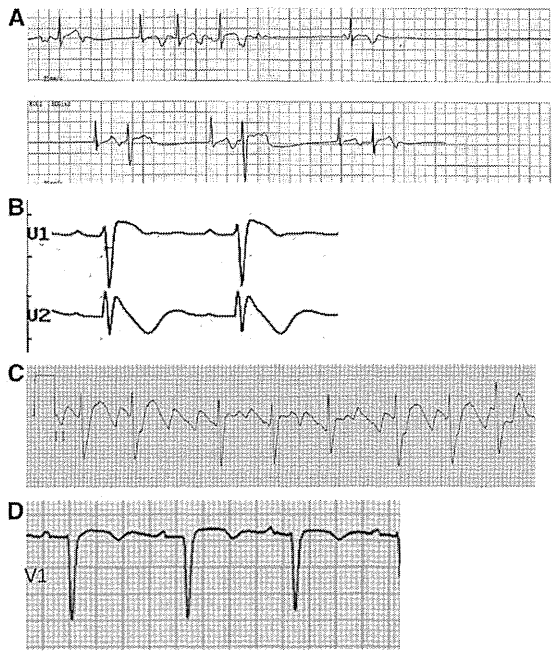

Figure 2. Electrocardiographic phenotypes. **A**, Consecutive strips of Holter ECG recording from proband A1-III:2 carrying compound heterozygous *SCN5A* mutations showed sinus arrest for 5.9 s (at the age of 5). **B**, His mother A1-II:2 showed coved-type ST-segment elevation in V1 through V2 leads during the flecainide challenge test. **C**, Paroxysmal atrial flutter (AFL) recorded in the proband A3-II:2. **D**, QT prolongation (QTc, 522 ms) remains evident in the proband A5-II:2 even after thyroid hormone supplemental therapy.

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 ≤6 V, compatible with atrial standstill. Genetic screening revealed 2 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) and heterozygous M1880V was observed in maternal family members (I:2, II:2, and III:3), demonstrating that the proband is a compound carrier of 2 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-segment elevation provoked by the Na channel blocker flecainide (Figure 2B). The remaining affected members were asymptomatic and had no sign of cardiovascular diseases.

Family A2

An 18-year-old woman (II:2) was admitted to hospital because of dizziness on standing. Holter ECG recording revealed frequent episodes of sinus arrest with a maximum R-R 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 a third-degree atrioventricular 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 beats per minute; maximum R-R interval=5 s) with left axis deviation. Echocardiography revealed dilatation of the left ventricle (left ventricular end-diastolic diameter, 59 mm), but the left ventricular 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]

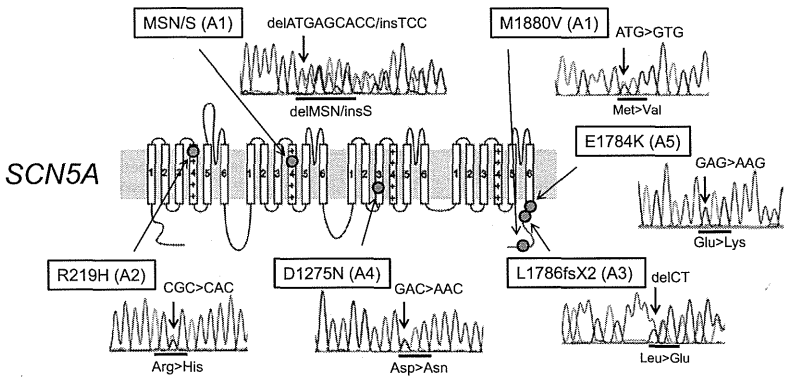

Figure 3. Sequencing of *SCN5A*. Schematic of the transmembrane topology of *SCN5A* representing the location and sequencing electropherogram of each mutation. Three mutations are located in the transmembrane domains, and 3 are in the cytoplasmic C-terminal.

The mutation was identified in his brother (III:1), 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), atrioventricular 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 3 family members carried an *SCN5A* missense mutation in exon 28, E1784K (c.5350C>A; Figure 3), which is the most common *SCN5A* mutation in type-3 LQTS (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 5 families with SSS (A1–A5; Table II in the Data Supplement). Seven individuals (37%) exhibited SSS, while 7 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 noninactivating late current, which characterizes LQT3 mutations,[24] was not evident. Peak current density measured 24 hours after transfection was significantly reduced in MSN/S, M1880V+MSN/S, R219H, and L1786fsX2 compared with wild type (Figure 4B and 4C). Because L1786fsX2 was nonfunctional, channel properties were further analyzed for M1880V+MSN/S and R219H (biophysical properties of other mutations are shown in Table III in the Data Supplement). 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 nonfunctional 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 less than that of 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 5 (A1–A5) in our cohort (Table IV in the Data Supplement). To our surprise, the 29 SSS probands with *SCN5A* mutations exhibited not only 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 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 6 *SCN5A* mutations in 15 familial SSS probands and demonstrated 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 Na channelopathy, including LQT3,[24] BrS,[25] progressive cardiac conduction defect,[26] sudden infant death syndrome, and SSS. To date, ≥27 distinct *SCN5A* mutations that are causative of SSS have been reported, although some mutation carriers exhibit mixed clinical phenotypes in addition to SSS (Table IV in the Data Supplement).[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 (Table III in the Data Supplement). 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.

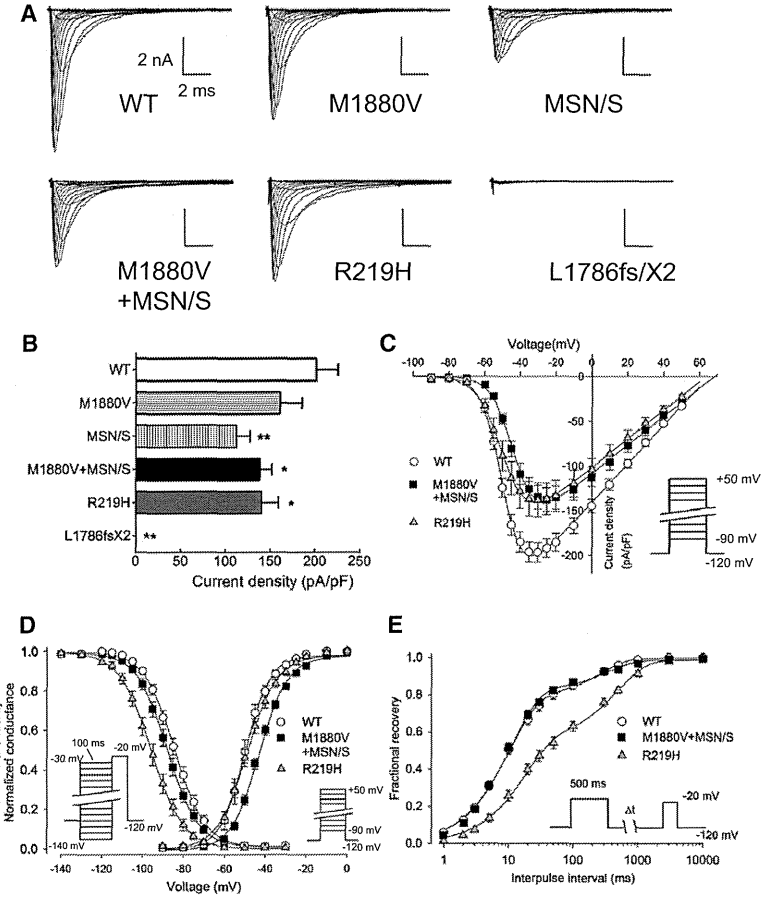

Figure 4. Whole-cell current recordings of wild-type (WT) and mutant Na channels. **A**, Representative whole-cell current traces obtained from tsA-201 cells transfected with WT or mutant Na channels. Currents were recorded from a holding potential of −120 mV and stepped to various membrane potentials from −90 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 ($P<0.05$), and in MSN/S and L1786fsX2 ($P<0.01$) compared with WT. **C**, Current–voltage relationship of WT and 2 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 Table III in the Data Supplement.

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 electric 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, Gosselin-Badaroudine et al[21] identified the R219H mutation in a family with DCM associated with ventricular tachycardia and a third-degree atrioventricular block. They found that the mutant R219H channel selectively permeates protons through the channel pore, which in turn results in severe left ventricular dysfunction and conduction disturbance. By contrast, clinical observations of our R219H carriers (A2-I:2, II:2) were rather benign, with electric abnormalities restricted to the sinus node with no left ventricular 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 Gosselin-Badaroudine et al[21] are not clear; however, additional genetic modifiers within *SCN5A* or other unidentified genes may contribute to the severe clinical and 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 nonfamilial SSS in the present study are elderly, with both sexes nearly equally affected (Figure 5). By contrast, our familial SSS cases associated with *SCN5A*

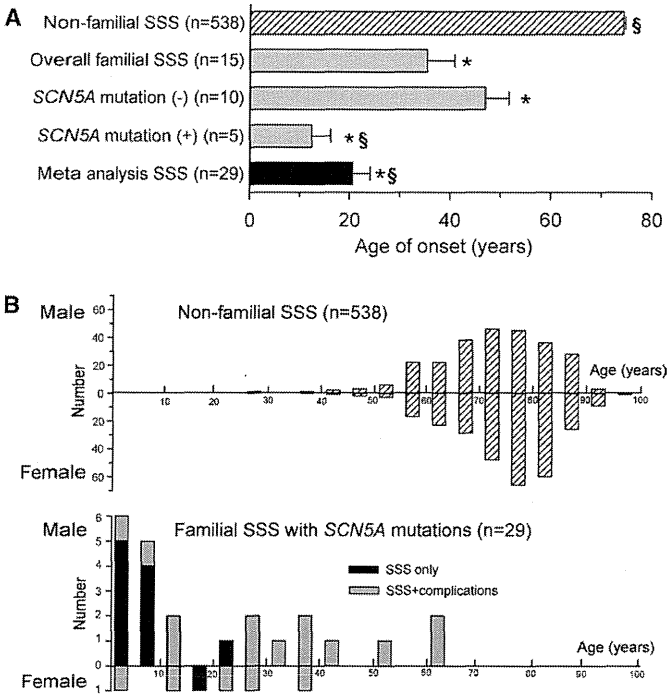

Figure 5. Age of onset and sex difference in probands with nonfamilial and familial sick sinus syndrome (SSS). **A**, Age of onset of nonfamilial SSS (n=538), SSS probands of our cohort (n=15) including *SCN5A* mutation negative (n=10) or positive (n=5), and meta-analysis of 29 cases with *SCN5A* mutations. § and * indicate $P<0.001$ vs mutation negative, and $P<0.001$ vs nonfamiliar SSS, respectively. **B**, Upper histogram shows age of onset in Japanese patients with nonfamilial SSS (n=538; 74.3±0.4 years): male (**upper**; n=241) and female (**lower**; n=257). There was no sex difference. Lower histogram shows the age of onset and sex 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.

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[11] genetically screened 38 BrS probands and identified 4 *SCN5A* mutation carriers (10.5%), all of which were complicated with bradyarrhythmias including SSS. 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 2 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 less (20.9±3.4 years) than those affected with BrS, which typically manifests during adulthood at a mean age of around 40 years. Furthermore, it should be noted that only 2 of 24 (8.3%) of the family members of our cohort who 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 (Table II in the Data Supplement), but longer term follow-up of the mutation carriers and 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.

Acknowledgments

We thank Saori Nakano and Atsuko Iida for technical assistance and attending physicians for referring SSS patients.

Sources of Funding

This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (High Definition Physiology) 22136007 (Dr Makita) and a Grant-in-Aid for Scientific Research (B) 24390199 (Dr Makita) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Disclosures

None.

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CLINICAL PERSPECTIVE

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. Recent studies have linked several genetic defects with familial SSS in genes including the cardiac Na channel (*SCN5A*); however, the pathogenesis and molecular epidemiology of familial SSS remain undetermined primarily because of its rarity. We genetically screened 48 members of 15 SSS families and identified 6 *SCN5A* mutations. Heterologously expressed mutant Na channels showed loss-of-function properties. Among 19 family members with *SCN5A* mutations, QT prolongation and Brugada syndrome were associated in 4 and 2 individuals, respectively. Age of onset in probands carrying *SCN5A* mutations was significantly less (12.4±4.6 years) than that in *SCN5A*-negative probands (47.0±4.6 years) or nonfamilial SSS (74.3±0.4 years). 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). 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. This study also suggests that SSS is the earliest electrophysiological manifestation of *SCN5A* mutation carriers, which may be associated with other arrhythmias such as long QT syndrome, Brugada syndrome, and dilated cardiomyopathy under the control of confounding factors including aging, hormones, other genetic variations, and undetermined environmental factors.

Correction

In the article "Sodium Channelopathy Underlying Familial Sick Sinus Syndrome With Early Onset and Predominantly Male Characteristics" by Abe et al, which was published in the June 2014 issue (*Circ Arrhythm Electrophysiol.* 2014;7:511–517), corrections were needed.

On page 514, left column, line 2, "brother (III:7)" should be "sister (III:7)."

For Figure 5B, a symbol of the 33-year-old male proband was erroneously missed in the figure. This individual corresponds to case 1 of the SSS with T187I mutation with BrS in the lower part of the Supplemental Table IV.

The authors apologize for the errors.

The online version of the article has been corrected.

(*Circ Arrhythm Electrophysiol.* 2014;7:771.)

***Circ Arrhythm Electrophysiol* is available at http://circep.ahajournals.org** **DOI: 10.1161/HAE.0000000000000006**

Journal of Arrhythmia ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Arrhythmia

journal homepage: www.elsevier.com/locate/joa

Review

The genetic background of arrhythmogenic right ventricular cardiomyopathy

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ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form
22 December 2015
Accepted 5 January 2016

Keywords:
Arrhythmogenic right ventricular cardiomyopathy
Desmosome
Genetic analysis
Mutation

ABSTRACT

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterized by degeneration of the right ventricle and ventricular tachycardia originating from the right ventricle. Additionally, the disease is an inherited cardiomyopathy that mainly follows the autosomal dominant pattern. More than 10 genes have been reported as causative genes for ARVC, and more than half of ARVC patients carry mutations in desmosome related genes. The desmosome is one of the structures involved in cell adhesion and its disruption leads to various diseases, including a skin disease called pemphigus. Among desmosome genes, mutations in PKP2 are most frequently identified in ARVC patients. Although the genotype–phenotype correlations remain to be fully studied, many studies have reported clinical manifestations of, prognosis for, and appropriate therapies for ARVC from the perspective of gene mutations. A collective review of these reports would enhance the understanding of ARVC pathogenesis and clinical manifestation. This review discusses the clinical issues of ARVC from the genetic background.

Contents

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http://dx.doi.org/10.1016/j.joa.2016.01.006

Please cite this article as: Ohno S. The genetic background of arrhythmogenic right ventricular cardiomyopathy. J Arrhythmia (2016), http://dx.doi.org/10.1016/j.joa.2016.01.006i

1. Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC), previously called arrhythmogenic right ventricular dysplasia (ARVD), is an inherited disease characterized by right ventricular degeneration and ventricular arrhythmias. ARVC is one of the important causes of sudden cardiac deaths in young people, and especially in young athletes [1]. The disease seems to be reported at the end of the 19th century as "cor adipose" [2]. In 1982, Marcus et al. first summarized 22 cases of adult ARVC patients, including their clinical characteristics such as male predominance, onset at around 40 years of age, T wave inversion in precordial leads, and fibro-fatty replacement of the myocardium [3]. These clinical characteristics are still applied in the latest diagnostic task force criteria [4].

Familial cases of ARVC have been reported since the early 1980s. In 1985, 3 out of 5 siblings from a family were diagnosed with ARVC, and the authors hypothesized incomplete autosomal dominant inheritance mode with low penetrance [5]. Thus, ARVC was suspected to be an inherited disease from the beginning, and many physicians and researchers started to explore the genes responsible for ARVC. Studies related to the identification of causative genes are summarized in the next section.

The first diagnostic criteria for ARVC published in 1994 included family history as one of the criteria [6]. Familial disease confirmed at necropsy or surgery was classified as a major criterion. A familial history of premature sudden death (< 35 years of age) due to suspected right ventricular dysplasia or a familial history based on clinically diagnosed disease as per the criteria were classified as minor criteria.

To understand the pathogenesis of ARVC, many researchers have studied the genetic background of the disease, and many causative genes have been identified in the last decade. Among these, the identification of involvement of desmosomal genes in ARVC patients was a significant discovery [7].

Presently, genetic mutations are identified in more than 60% of ARVC patients, and familial cascade screening is useful to diagnose the disease before its onset in young family members.

In this review, I have described the causative genes, the characteristics of the genotype, and the future perspectives for ARVC from the viewpoint of genetics.

2. History of desmosome genes as the cause of ARVC

Desmosomes are a complex formed by proteins and function to bind the myocardial cells to each other. In the heart, desmosomes are composed of five proteins that is, junctional plakoglobin encoded by JUP, plakophilin-2 by PKP2, desmoplakin by DSP, desmoglein-2 by DSG2, and desmocollin-2 by DSC2 (Fig. 1). Desmosomes are indispensable for electrical conduction and mechanical contraction in myocardial cells.

In 1986, the Greek cardiologist Protonotarios and his colleagues reported cardiac abnormalities in 9 patients with familial palmoplantar keratosis and wooly hair [8]. All patients originated from families on the Greek island of Naxos, and therefore the disease was named as Naxos disease. The island of Naxos has nearly 200 unrelated families. Medical doctors and scientists in Naxos and London collaborated and recruited all families living on the island of Naxos for the study. They identified 9 affected families and performed linkage analysis for 38 members, including the 14 affected members. Using this analysis, they reported a homozygous genotype on 17q21 in 1998 [9].

In 2000, a homozygous deletion mutation in JUP was finally identified in 19 patients with Naxos disease [7].

After the discovery of JUP as a causative gene for ARVC in the recessive form, many researchers started genetic analysis for other desmosome genes in ARVC patients. DSP was confirmed as a causative gene in 2002 [10]. PKP2 mutations in ARVC patients were reported in 2004 [11], while DSG2 and DSC2 mutations were reported in 2006 [12,13].

Although most ARVC patients show the autosomal dominant inheritance, two recessive inheritance modes have been reported in syndromic ARVC. One of these is the Naxos disease caused by homozygous mutations in JUP [7], and another is the Carvajal syndrome caused by homozygous DSP mutations [14]. At first, Carvajal syndrome was reported as a syndromic dilated

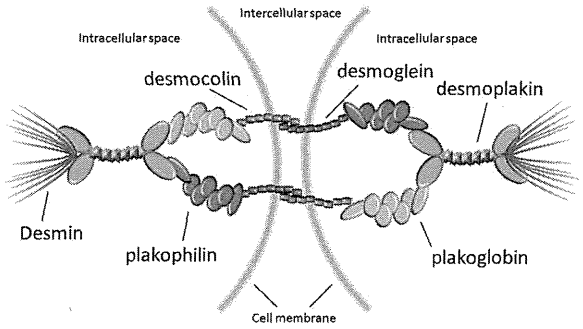

Fig. 1. A schematic diagram of the desmosome. Desmoglein and desmocolin located in the transmembrane region connect with the corresponding molecules on the neighboring cell and are linked to desmoplakin by plakophilin and plakoglobin.

Table 1
Genotypes, gene names, and locations related to ARVC.

Genotype	Gene	Location	Recessive form	Reference
ARVC1	*TGFB3*	14q24.3		[24]
ARVC2	*RYR2*	1q43		[27]
ARVC3	Unknown	14q12-q22		[54]
ARVC4	*TTN*	2q32.1-q32.3		[29]
ARVC5	*TMEM43*	3p25.1		[33]
ARVC6	Unknown	10p14-p12		[55]
ARVC7	*DES*	2q35		[37]
ARVC8	*DSP*	6p24.3	Carvajal syndrome	[10]
ARVC9	*PKP2*	12p11		[11]
ARVC10	*DSG2*	18q12.1		[12]
ARVC11	*DSC2*	18q12.1		[13]
ARVC12	*JUP*	17q21.2	Naxos disease	[7]
Others	*PLN*	6q22.1		[40]
	LMNA	1q22		[41]
	SCN5A	3p21		[44]
	CTNNA3	10q 22.2		[45]

Please cite this article as: Ohno S. The genetic background of arrhythmogenic right ventricular cardiomyopathy. J Arrhythmia (2016), http://dx.doi.org/10.1016/j.joa.2016.01.006i

cardiomyopathy with woolly hair and keratoderma caused due to a homozygous mutation in DSP [14]. In 2003, another homozygous mutation in DSP was identified in an ARVC family showing hair and skin abnormalities [15].

Causative genes for ARVC and their respective characteristics are summarized in Table 1.

3. Desmosome genes for ARVC

3.1. PKP2

PKP2 is the most major causative gene for ARVC. PKP2 encodes plakophilin-2, a protein with 881 amino acids with armadillo repeat domain (Fig. 1) and has a structure similar to that of plakoglobin, which is encoded by JUP. In 2004, PKP2 mutations were identified in 32 out of 120 unrelated individuals with ARVC [11]. Later studies demonstrated the low penetrance of PKP2 mutations in ARVC [16,17]. Among the carriers of mutations in desmosome genes, more than 70% patients in western countries carry PKP2 mutations [18]. In Asian countries, we have previously reported the results of genetic analysis in 35 ARVC probands [19]. Among 35 probands, we identified 19 carriers with desmosome mutations, with 10 showing mutations in PKP2 (52.6%). In China, Bao et al. identified 57 mutation carriers from 90 ARVC patients (63.3%); of these, 40 were PKP2 mutation carriers (70.2%) [20]. These reports indicate that genetic screening for PKP2 in ARVC patients is indispensable to understand their genetic backgrounds.

3.2. JUP

JUP was the first gene identified as causative for ARVC among the desmosome genes [7], and the structure of plakoglobin, which is encoded by JUP, is similar to that of plakophilin (Fig. 1). However, the mutation frequency of JUP in ARVC patients is low as compared to that of other desmosome genes. The number of JUP mutations identified in ARVC patients is less, but plakoglobin encoded by JUP has a remarkable effect in ARVC pathogenesis. In an experiment where desmoplakin expression in atrial myocyte cell lines was suppressed by siRNA, nuclear translocation of plakoglobin and reduction of Wnt/beta-catenin signaling were reported [21]. In 2009, translocated plakoglobin to nuclear was shown to bind the Tcf7l2 transcription factor, resulting in increased expression of adipogenic factors like Wnt5b and BMP7, which are normally inhibited by canonical Wnt signaling [22]. Therefore, JUP-encoded plakoglobin could be a key factor for dissolving the pathogenesis of ARVC.

3.3. DSP

DSP is the largest among the desmosome genes and encodes the 2872 amino acid protein, desmoplakin. The N-terminus of desmoplakin is required for localization to the desmosome and interaction with plakophilin and plakoglobin. The C-terminus of desmoplakin binds to the intermediate filaments (desmin) (Fig. 1). A DSP mutation was first identified in a homozygous manner as the cause of Carvajal disease, which shows dilated cardiomyopathy with wooly hair and keratoedema [14]. In 2002, a DSP mutation, S229R, was identified in a 18 year-old male who suffered cardiac arrest and was diagnosed with ARVC [10]. An extended clinical and genetic analysis of his family members from 4 generation confirmed that the mutation was the cause of ARVC. The residue 229 located in the N-terminus of desmoplakin is involved in binding with plakoglobin or plakophilin, and the mutation S229R would disrupt the normal binding with those proteins.

After the first report, other DSP mutations have been identified in ARVC patients. Although DSP is the largest among the desmosome genes, the number of reported mutations is small compared to other genes. Only 12 mutations have been reported in recent study with 439 families [18].

3.4. DSG2 and DSC2

The products of DSG2 and DSC2 are cadherin-like transmembrane glycoproteins that are major components of the desmosome (Fig. 1). Mutations in both genes have been reported as the cause of ARVC.

Mutations in DSG2 as a causative gene for ARVC were first reported in 2006 [12]. Among 54 ARVC probands who were negative for mutations in DSP, PKP2, and TGFβ3, 9 heterozygous DSG2 mutations were found.

DSC2 was the last one among the desmosome genes to be reported as the causative gene for ARVC. Among 77 probands who were negative for PKP2, JUP, DSP and DSG2, two frameshift mutations that resulted in the formation of a premature termination codon in DSC2 were identified from 4 families [13].

After these reports, many mutations in DSG2 and DSC2 have been reported. However, in European countries, the frequency of mutations in these genes is low compare to PKP2. In a recent report, 17 DSG2 (4%) and 5 DSC2 (1%) mutation carriers were identified from 276 genotype positive probands [18].

In Asian countries, the frequency of DSG2 mutations is rather higher than in Caucasians. We reported 3 DSG2 (15.8%) mutation carriers from 19 genotype positive patients [19] and Bao et al. reported 8 DSG2 (14%) mutation carriers from 57 patients [20]. Surprisingly, the latter reported that 3 of the 8 DSG2 mutation carriers had homozygous mutations.

DSC2 mutations in ARVC patients are also rare in Asian countries. We identified only one DSC2 mutation carrier from 19 genotype positive patients; the patient carried three DSC2 mutations; R132C, N194K, and R203C [19]. In China, 3 DSC2 mutations were identified from 57 patients, and two of these mutations were identified as a single mutation [20].

4. Other causative genes for ARVC

Although more than half of ARVC patients carry mutations in desmosome genes, other genes have also been reported as causative genes for ARVC (Table 1). In the most of these genes, their loci on chromosomes were first confirmed by linkage analysis, and then the specific genes were identified in the target families. Other than desmosome genes, 7 loci have been detected in linkage analysis and 5 causative genes for ARVC have been identified. However, the causative genes of ARVC3 in chromosome 14q12-q22 and ARVC6 in chromosome 10q14-p12 have not been identified yet.

4.1. ARVC1-TGFB3

The ARVC1 locus on chromosome 14q23-q24 was first identified in 1994 as the causative locus for ARVC on performing linkage analysis of two families, including a large family with 4 generations of ARVC patients [23]. However, identification of the causative gene, TGFB3, took a lot of time. In 2005, two nucleotide substitutions in the 5′UTR and 3′UTR of TGFB3 were identified in ARVC patients [24]. To confirm the effect of UTR mutations in TGFB3 gene expression, a luciferase reporter assay was performed, and both UTR mutations were found to increase the luciferase reporter activity. Thus, the authors explained that increased TGFB3 expression induced myocardial fibrosis in accordance with previous studies [25].

Please cite this article as: Ohno S. The genetic background of arrhythmogenic right ventricular cardiomyopathy. J Arrhythmia (2016), http://dx.doi.org/10.1016/j.joa.2016.01.006i

4.2. ARVC2-RYR2

RYR2 encodes the cardiac ryanodine receptor, which locates to the sarcoplasmic reticulum, and is indispensable for cardiac contraction by controlling the calcium ions. The RYR2 mutations were reported in various other cardiac diseases, including dilated cardiomyopathy (DCM) and catecholaminergic polymorphic ventricular tachycardia (CPVT). The locus, 1q42-q43 was confirmed in an ARVC family in 1995 [26] and the causative gene was identified as RYR2 in 2001 [27]. The RYR2 mutation carriers in this family showed effort-induced polymorphic ventricular tachycardia, and there were four patients of juvenile sudden death in the family. Post mortem examination of two subjects showed normal right ventricular size, but large areas of fatty-fibrous replacement localized in the sub-epicardial layer of the right ventricle were detected at the histological level [26]. In 2001, four RYR2 mutations (R176Q, L433P, N2386I, and T2504M) from three ARVC families were identified [27]. Although the affected family members fulfilled the diagnostic criteria of ARVC at that time [6], this ARVC family with RYR2 mutations might actually be a variant of CPVT.

4.3. ARVC4-TTN

ARVC4 was reported in three families with left ventricular involvement in1997 [28], and TTN was confirmed as the causative gene for ARVC4 in 2011 [29]. TTN consists of 363 exons and encodes the protein, titin, which is the largest protein in mammals, with its name originating from the giant men in Greek mythology. TTN mutations have been reported in other cardiomyopathies such as hypertrophic cardiomyopathy (HCM) [30] and dilated cardiomyopathy (DCM) [31]. ARVC4 patients with TTN mutations displayed various phenotypes, including biventricular dysfunction and conduction block. Therefore, overlapping phenotypes with DCM are suspected in ARVC4 patients.

4.4. ARVC5-TMEM43

The locus of ARVC5 (chromosome 3p23) was detected in a large ARVC family from the island of Newfoundland including more than 200 members spanning eight generations [32]. In 2008, the causative gene of ARVC5 was confirmed as TMEM43 encoding the transmembrane protein 43, which functions as a nuclear membrane organizer [33]. The mutation identified in the large family was S358L located in the transmembrane domain of the protein. Mutations in TMEM43 were also identified in 2 of 41 unrelated individuals with Emery–Dreifuss muscular dystrophy (EDMD) [34]. As the cause of ARVC, TMEM43 mutations were regarded as rare compared to desmosome mutations. However, recent advances in genetic analysis have made it easy to identify TMEM43 mutations in ARVC patients. Recently, we identified double TMEM43 mutations in a Japanese family with RV aneurysm [35].

Although ARVC patients with TMEM43 mutations fulfill the ARVC task force criteria, the phenotype of ARVC due to TMEM43 mutations might be different from that due to desmosome mutations. One of these differences includes the early onset of ARVC. In general, ARVC onset occurs at around 30 years of age, but in our patient with TMEM43 mutation, RV aneurysm and ventricular arrhythmia were identified before birth by fetal ultrasound [35]. In addition, the penetrance of TMEM43 related ARVC is very high. In an analysis of 137 TMEM43 mutation carriers, all the patients showed ARVC specific phenotype by 63 years in males and 76 years in females [33].

4.5. ARVC7-DES

The locus of ARVC7 was first detected on chromosome 10q [36], but in 2010, ARVC7 was determined to be caused by DES located on chromosome 2q35 [37]. DES encodes desmin, which is one of the types of intermediate fibers in myocytes (Fig. 1) and is related to myofibrillar myopathies. The patients discussed in the first report of ARVC7 had myopathy and cardiomyopathy [36]. In 2010, an ARVC7 patient without myopathy was reported [37]. The mutation frequency of DES is not high, but in the screening of 91 ARVC probands, 2 DES mutation carriers were identified [38]. One of the patients carried a PKP2 mutation in addition to a rare variant of DES, whereas the other patient carried a frameshift DES mutation. Therefore, the mutation frequency of DES seems to be more than 1%.

5. Other causative genes for ARVC

By using candidate gene sequence methods instead of linkage analysis, other causative genes for ARVC have been reported.

5.1. PLN

PLN encodes phospholamban, which is indispensable for calcium handling in cardiac contractions [39] and was known as the causative gene for DCM. In 2012, van der Zwaag et al. identified PLN-R14del in 12 out of 97 ARVC and 39 out of 257 DCM patients in the Netherlands [40]. However, no PLN mutations except for R14del have been reported in ARVC patients.

5.2. LMNA

LMNA encodes lamin A/C, which functions in the lining of the nuclear membrane and is indispensable for stabilization of cells. LMNA mutations were reported in various systemic diseases, including Emery–Dreifuss muscle dystrophy and premature aging syndrome. In the cardiovascular field, LMNA mutations were identified in DCM patients, especially those with sinus bradycardia and conduction disturbance. In 2011, four LMNA mutations in ARVC patients were reported [41]. Two of the four patients died suddenly at ages 54 and 67, and one died from severe heart failure at the age of 48 years. The histological characteristics of one of the patients were compatible with those of ARVC, including fibro-fatty replacement. Recently, we reported two ARVC families with LMNA mutations [42]. ARVC probands in both families showed bradycardia and were implanted with pacemakers.

5.3. SCN5A

SCN5A encodes the cardiac sodium channel, and mutations in SCN5A cause various cardiac diseases, including long QT syndrome type 3, Brugada syndrome, progressive cardiac conduction disease, and DCM [43]. In 2008, an ARVC patient with a SCN5A splice variant, c.3840+1g>a, was reported, and frequent VT was recorded in his ICD [44].

5.4. CTNNA3

CTNNA3 is the newest candidate gene for ARVC [45]. Alpha-T-catenin encoded by CTNNA3 binds with plakophilins and functions in the cell–cell adhesion in cardiomyocytes. Two CTNNA3 mutations, c.281t>a (p.V94D) and c.2293_2295delTTG (p.dl765L) were identified in 2 out of 76 ARVC patients without any mutations in desmosome genes. In functional analysis, CTNNA3-V94D showed disabled interaction with β-catenin, and CTNNA3-del765L showed

Please cite this article as: Ohno S. The genetic background of arrhythmogenic right ventricular cardiomyopathy. J Arrhythmia (2016), http://dx.doi.org/10.1016/j.joa.2016.01.006i

much stronger dimerization potential. These functional changes suggest a causal relationship between CTNNA3 mutations and ARVC pathology.

6. Genotype–phenotype correlations

In certain genetic diseases with multiple genotypes, genotype–phenotype correlations are well studied and used for diagnosis and treatment. For example in long QT syndrome, phenotypes predict the genotypes, and genotyping is useful to predict prognosis of the patients and to decide treatment. However, in ARVC, genotype–phenotype correlations have not been fully examined as of yet. One of the reasons being that most of the mutations are identified in PKP2 [18]. Another reason for the lack of study is the low penetrance of the mutations [17].

Recently, a study that analyzed the phenotypes and gene mutations related to ARVC was reported. In the probands, mutation positive status did not affect clinical characteristics and outcomes. In contrast, family members with mutations were more likely to meet Task Force Criteria for ARVC [4] (40% vs 18%), experience sustained ventricular arrhythmias (11% vs 1%), and die from a cardiac cause (2% vs 0%) than family members without mutations [18]. Among 116 desmosome gene mutation carriers in another study, the event rate was higher among patients with definite ARVC than among borderline or phenotype negative patients [46].

Correlations between mutation type and phenotype have been reported. We compared the disease onset of probands with 12 missense and 7 non-missense mutation carriers. All non-missense mutations were found in PKP2 [19]. In our study, the disease onsets were significantly younger in the patients with non-missense mutations than in those with missense mutations (29.4 ± 12.4 vs 45.8 ± 14.2 years, $P=0.027$). In contrast, Alcalde et al. reported that the disease onsets in patients with stop gain mutations were later than those in patients with missense mutations (27 vs 39 years old, $P < 0.05$). Although, it is difficult to clarify the reason of the discrepancy, the definition of onset was different between studies. It would be difficult to define disease onsets in the patients whose first symptoms were ventricular arrhythmias, because cardiologists would never recognize that the patients experienced ventricular tachycardia if the patients did not complain of their symptoms or had syncope.

7. Progress pertaining to genetic analysis and genetic noise

At present, the causative genes for ARVC are known, and we can screen all the genes in patients who are diagnosed with ARVC. The gold standard of sequencing for genetic screening is the Sanger method, which needs two times of PCR for target exons. All the target exons can be screened in genes related to ARVC. However, Sanger methods need a long time and are expensive if we screen all the exons in those genes. The total number of exons in desmosome genes is nearly 90. In addition, TTN, a causative gene for ARVC4, has 363 exons, and RYR2, a causative gene for ARVC2, has 105 exons. The screening of all ARVC-related genes by Sanger methods therefore seems to be a difficult task.

However, we can now approach the new sequencing methods, next generation sequencing (NGS) methods [47]. These methods are useful not only for whole genome or exome sequencing but also for target gene sequencing [48]. Especially for cardiomyopathies, multiple panels for genetic analysis are available on the market. If these panels are used, mutations in ARVC patients can easily be detected in a week.

In the NGS era, many rare variants can be detected in genetic analysis, some of which are related with the disease. Therefore, the true mutation(s) causative for the disease need to be distinguished from other genetic noise. In 2011, Kapplinger et al. reported background genetic noise in ARVC [49]. They performed a genetic analysis of PKP2, DSP, DSG2, DSC2, and TMEM43 for 92 probands diagnosed with ARVC and 427 controls. Radical mutations resulting in stop codons were identified in 43% of probands and in 0.5% of controls. In contrast, missense mutations were identified in 21% of the probands and 16% of controls; thus, the frequency of missense mutations was similar between probands and controls. They further analyzed the ARVC related genes and locations of missense mutations that were highly detected in controls and found that missense mutations in DSP and DSG2, especially at the C-terminals of both genes, were highly detected in controls [49]. To avoid misunderstanding of the detected variants in the ARVC related genes, a comparison of the detected mutations with data for ethnically matched controls is needed, for example, the 1000 genome study [50] and the ExAC Browser (http://exac.broadinstitute.org/).

In silico prediction software, SIFT [51], PolyPhen2 [52], CADD [53], and other prediction systems are useful to evaluate a specific mutation which is detected in a patient. However, even if the in silico software predicts a mutation as pathogenic, we need to examine whether the genetic result is compatible with the phenotype.

8. Conclusion

In the last decade, there has been remarkable progress in determining the genetic background of ARVC and new technologies for genetic analysis have been developed. The next step is to utilize this genetic progress for the treatment of ARVC, including the prevention of sudden cardiac death in young people.

Conflict of interest

All authors declare no conflicts of interest related to this study.

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Please cite this article as: Ohno S. The genetic background of arrhythmogenic right ventricular cardiomyopathy. J Arrhythmia (2016), http://dx.doi.org/10.1016/j.joa.2016.01.006i

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Please cite this article as: Ohno S. The genetic background of arrhythmogenic right ventricular cardiomyopathy. J Arrhythmia (2016), http://dx.doi.org/10.1016/j.joa.2016.01.006i

European Heart Journal Advance Access published December 28, 2015

European Heart Journal
doi:10.1093/eurheartj/ehv695

CLINICAL RESEARCH
Arrhythmia/electrophysiology

The genetics underlying acquired long QT syndrome: impact for genetic screening

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Received 25 February 2015; revised 18 November 2015; accepted 1 December 2015

Aims Acquired long QT syndrome (aLQTS) exhibits QT prolongation and Torsades de Pointes ventricular tachycardia triggered by drugs, hypokalaemia, or bradycardia. Sometimes, QTc remains prolonged despite elimination of triggers, suggesting the presence of an underlying genetic substrate. In aLQTS subjects, we assessed the prevalence of mutations in major LQTS genes and their probability of being carriers of a disease-causing genetic variant based on clinical factors.

Methods and results We screened for the five major LQTS genes among 188 aLQTS probands (55 ± 20 years, 140 females) from Japan, France, and Italy. Based on control QTc (without triggers), subjects were designated 'true aLQTS' (QTc within normal limits) or 'unmasked cLQTS' (all others) and compared for QTc and genetics with 2379 members of 1010 genotyped congenital long QT syndrome (cLQTS) families. Cardiac symptoms were present in 86% of aLQTS subjects. Control QTc of aLQTS was 453 ± 39 ms, shorter than in cLQTS (478 ± 46 ms, $P < 0.001$) and longer than in non-carriers (406 ± 26 ms, $P < 0.001$). In 53 (28%) aLQTS subjects, 47 disease-causing mutations were identified. Compared with cLQTS, in 'true aLQTS', *KCNQ1* mutations were much less frequent than *KCNH2* (20% [95% CI 7–41%] vs. 64% [95% CI 43–82%], $P < 0.01$). A clinical score based on control QTc, age, and symptoms allowed identification of patients more likely to carry LQTS mutations.

Conclusion A third of aLQTS patients carry cLQTS mutations, those on *KCNH2* being more common. The probability of being a carrier of cLQTS disease-causing mutations can be predicted by simple clinical parameters, thus allowing possibly cost-effective genetic testing leading to cascade screening for identification of additional at-risk family members.

Keywords Congenital long QT syndrome • Acquired long QT syndrome • Drug-induced long QT syndrome • Genetics

Introduction

Acquired long QT syndrome (aLQTS) is characterized by QT prolongation and sometimes Torsades de Pointes (TdP) ventricular tachycardia, and is provoked by the presence of QT-prolonging drugs,[1,2] hypokalaemia,[3] or bradycardia.[4] Culprit agents in aLQTS include many in common use, such as antihistamines, antibiotics, antidepressants, prokinetics, and many others.[1] The importance of

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aLQTS depends on its two main consequences: the unacceptable risk of sudden death when being medically treated for a non-life-threatening condition, and the implications for new drug development as many potentially useful agents are withdrawn from the market following a small number of cases of TdP.[1] The problem of drug-induced aLQTS has not been solved despite the efforts made by the Food and Drug Administration,[5–7] including strict non-clinical (S7B) and clinical (ICH E14) tests.

While, in most aLQTS cases, the QT interval returns to normal after the discontinuation of drug or other causative trigger, in some cases it remains prolonged. Partly on this basis, since 1982[8] it had been suggested that aLQTS may share some genetic background with congenital long QT syndrome (cLQTS).[9–11] In LQTS, 16 disease-causing genes have been identified so far, mostly encoding cardiac ion channel subunits or associated proteins.[12] Individual genetic background could be important, even in the presence of modest QT prolongation, because in association with appropriate triggers, it may favour the life-threatening manifestations of aLQTS. In this international collaborative study, we analysed a cohort of 188 aLQTS cases, assessed the prevalence of mutations in the major LQTS genes when compared with that in 1010 cLQTS probands, and evaluated the association of clinical factors, including QTc, with the probability of aLQTS subjects being carriers of a disease-causing genetic variant.

Methods

Study population

A total of 188 subjects diagnosed with aLQTS were identified at the participating centres in France ($n = 20$), Italy ($n = 21$), and Japan ($n = 147$). Patients were considered symptomatic if they exhibited TdP ventricular tachycardia, pre-syncope, syncope, cardiac arrest, or ventricular fibrillation, or as asymptomatic if they had a prolonged QTc (QT interval corrected for heart rate according to Bazett's formula[13] $\geq$480 ms) in the presence of proarrhythmic determinants.

QTc measurement

The QTc of aLQTS was measured in the presence and absence ('control QTc') of triggering factors. Control QTc was recorded either before or after an adequate period of time had elapsed from the critical event to allow washout and/or correction of the triggering condition. Depending on the value of control QTc, aLQTS subjects were retrospectively divided into two groups: 'true aLQTS' (QTc <460 ms in females and <450 ms in males) or 'unmasked cLQTS' (all non-true aLQTS). Control QTc in aLQTS was also compared with that of 2379 genotyped members (1938 carriers and 441 non-carriers) of 1010 cLQTS (Romano-Ward) families recruited in Japan ($n = 435$), France ($n = 296$), and Italy ($n = 279$).

Genetic analysis

The protocol for genetic analysis was approved by and performed under the guidelines of each institutional Ethics Committee. Written informed consent to participate in the study including consent for the collection and the use of DNA samples for genetic analysis was obtained in each centre. Genomic DNA was isolated from peripheral white blood cells using conventional methods. Genetic screening was performed for *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* genes, corresponding to LQT1, LQT2, LQT3, LQT5, and LQT6, in each centre. All index cases were tested; family members were analysed only if the proband had a mutation. We considered as disease-causing those mutations for which a link with LQTS has already been established in previous publications. In the absence of references, novel variants were included when deemed to be pathogenic or likely pathogenic applying the recently published ACMG guidelines,[14] which are based on criteria using typical types of variant evidence, including frequencies in population data and computational (*in silico*) predictive programmes. Two KCNH2 novel variants of uncertain significance according to ACMG criteria were included because they had never been described in any control population and both were present in the same subject, making very unlikely that such combined heterozygosity had not contributed to the clinical manifestations. Common polymorphisms and rare variants in accordance with previous reports were excluded.[15,16] Nevertheless, given its role as a QT modulator and a risk factor in cLQTS and aLQTS,[17,18] we also examined the frequency of the common variant *KCNQ1*-D85N.

Scoring system

We developed and assessed the performance of a composite score in predicting the probability of being a mutation carrier. Univariate analyses were first used to derive those demographic/clinical variables shown to be significantly associated with the presence of a disease-causing mutation. Three significant ($P < 0.05$) variables were identified, i.e. age, QTc, and the presence of symptoms, which were then included in a multivariable logistic regression model with the genetic status [mutation carriers (MCs)/non-mutation carriers (NMCs)] as the dependent variable. These three predictors were treated as dichotomized variables: for age at the critical event, the predefined cut-off was 40 years, the usual time horizon for the exposure to the risk of a first cardiac event in cLQTS; for the QT interval, the cut-off was 440 ms, the traditional limit for ECG-based diagnosis of cLQTS; the third variable was the presence/absence of symptoms at the time of aLQTS diagnosis. The positive presence of each variable was specifically categorized (i.e. age <40 years; QTc >440 ms; history of cardiac events) conferred 1 point. Thus, the score made up by adding the points for each factor present ranged from 0 to 3 points. We also considered the relative magnitude of the specific contribution of the three predictors and tested in a sensitivity analyses a 'weighted' version of the score. Specifically, points were derived from the final logistic regression model beta-coefficients and were assigned to each factor by dividing each beta coefficient by the smallest beta coefficient in the model and rounding to the nearest integer. Thus, this weighted scoring system assigned 1 point for age <40 years, 2 points for a QTc >440, and 3 points for a history of symptoms. As a result, each patient received a score ranging from 0 to 6 points. The goal was to identify a simple composite indicator of the probability of the presence of an LQTS mutation, based on features easily available to clinicians.

Statistical analysis

Normal continuous variables, expressed as mean $\pm$ standard deviation (SD), were compared by an unpaired *t*-test or by one-way ANOVA. Categorical variables, expressed as numbers and percentages, were analysed by χ^2 or Fisher's exact test, as appropriate. Binomial exact 95% confidence intervals (CIs) were computed to assess the reliability of the estimated proportions of mutation carriers. Multivariable logistic regression analysis was performed to evaluate the independent contribution of demographic and clinical variables to the probability of being mutation carriers among aLQTS individuals. All variables were tested for collinearity to exclude over-correlation with one another. Odds ratios with 95% CI are reported. To assess the discriminating power of the scoring system, i.e. its performance in distinguishing between aLQTS subjects likely to be or not mutation carriers, we used the receiving operator characteristic curve. A value of $P < 0.05$ was considered

statistically significant. SPSS Statistics version 21 (IBM Co, Armonk, NY, USA) was used for computation.

Results

Clinical background of acquired long QT syndrome

The larger aLQTS Japanese subset ($n = 147$) was not significantly different compared with the Caucasian one ($n = 39$) in all measured baseline clinical features, with the only exception of a somewhat older age. Among all the 188 aLQTS patients, females ($n = 140$, 74.5%) were prevalent and the mean age at the critical event was 55 ± 20 years. Most subjects ($n = 162$, 86%) were symptomatic; the remaining 26 (14%) came to medical attention because of a marked QT prolongation associated with secondary factors. These included: drugs ($n = 81$, Supplementary material online, *Table S1*), hypokalaemia (serum level <3.5 mEq/L, $n = 42$), bradycardia (mostly sick sinus syndrome or atrioventricular block, $n = 17$), a combination of at least two factors ($n = 43$), and 'other' factors ($n = 5$, three Takotsubo cardiomyopathy, one hypothermia, and one subarachnoid haemorrhage). When a distinction was made between drug-induced LQTS and all other causes of aLQTS, the two groups were comparable with respect to almost all baseline characteristics, the only exception being a significantly larger proportion of symptomatic and mutation carriers among those individuals with hypokalaemia, bradycardia, and 'other' factors as aLQTS determinants (*Table 1*). When aLQTS was secondary to hypokalaemia, the mean serum potassium concentration was 2.8 ± 0.5 mEq/L.

Latent QT prolongation in acquired long QT syndrome

The mean control QTc of the 188 aLQTS patients was 453 ± 39 ms, significantly shorter than that of the 1938 cLQTS MCs (478 ± 46 ms, $P < 0.001$) but longer than that of the 441 NMCs (406 ± 26 ms, $P < 0.001$). This suggests that already in the absence of any triggering factor, there is latent QTc prolongation (*Figure 1*). Based on their control QTc, 112 (60%) patients were true aLQTS and 76 (40%) were unmasked cLQTS. *Figure 2* shows representative ECGs of one patient of each subgroup. In association with the induced critical event, the QTc of aLQTS patients was dramatically prolonged (591 ± 82 ms).

Genetic background in acquired long QT syndrome

Genetic analysis identified 51 carriers of a single LQTS disease-causing mutation and 2 compound heterozygous carriers of genetic variants in different genes (*KCNQ1*/*KCNH2*) or in the same gene (*KCNH2*/*KCNH2*). Overall, 53 of the 188 aLQTS cases (28%, 95% CI 22–35) were found to be MCs following accidental exposure to an arrhythmia-triggering factor. A detailed list of all the 47 distinct mutations identified is presented in Supplementary material online, *Table S2*. Of these (all previously reported with the exception of 9 novel mutations), 13 were found in *KCNQ1*, 29 in *KCNH2*, 3 in *SCN5A*, 1 in *KCNE1*, and 1 in *KCNE2*. Mutations were found regardless of ethnicity, sex, or triggers, but were more frequently found in aLQTS patients diagnosed before age 40 than afterwards (41 vs. 24%, $P = 0.03$) and in symptomatic than in asymptomatic aLQTS (32 vs. 4%, $P = 0.002$; *Table 2*). Control QTc of the 53 aLQTS MCs was significantly longer than that observed in the 135 aLQTS NMCs (469 ± 36 vs. 447 ± 38 ms, $P < 0.001$), but comparable to the control QTc measured in the cLQTS MCs (478 ± 46 ms). Conversely, the QTc of aLQTS NMCs (447 ± 38) was more prolonged than that of cLQTS NMCs (406 ± 26, $P < 0.001$).

The analysis of aLQTS family members identified 56 mutation carriers (37 ± 24 years), with a mean QTc of 459 ± 36 ms. Of these 56 MCs, 54 (96%) were asymptomatic while 2 females with the R555C mutation in *KCNQ1* or the M124T mutation in *KCNH2* had

Table 1 Comparison between drug-induced LQTS and aLQTS from other causes

	Drug-induced aLQTS ($n = 117$)[a]	Non-drug-induced aLQTS ($n = 71$)	*P*-value
Females	83 (71)	57 (80)	0.15
Age (years)	55 ± 22	54 ± 19	0.64
QTc off trigger (ms)	451 ± 39	455 ± 39	0.49
True aLQTS	69 (59)	43 (61)	0.83
Unmasked LQTS	48 (41)	28 (39)	
QTc on trigger	585 ± 85	601 ± 76	0.23
Japanese : Caucasian : Black	91 (78) : 24 (20) : 2 (2)	56 (79) : 15 (21) : 0 (0)	0.54
Symptomatic	94 (80)	68 (96)	0.003
Mutation carriers	27 (23)	26 (37)	0.045
KCNQ1	9 (33)	6 (23)	0.167
KCNH2	13 (48)	17 (65)	
SCN5A	3 (11)	0	
KCNE1/*KCNE2*	2 (7)	1 (4)	
Double mutations	0	2 (8)	

Data are frequencies (%) or mean ± SD.
LQTS, long QT syndrome; aLQTS, acquired long QT syndrome.
[a]QT drugs only ($n = 81$) and QT drugs in association with hypokalaemia ($n = 27$) or bradycardia ($n = 9$).