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Congenital Atrial Standstill Associated with Coinheritance of a Novel *SCN5A* Mutation and Connexin 40 Polymorphisms

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ABSTRACT

OBJECTIVES: The purpose of the study was to determine the clinical and biophysical characteristics of a novel *SCN5A* mutation identified in a family with atrial standstill.

BACKGROUND: Congenital atrial standstill has been linked to *SCN5A*. Incomplete penetrance observed in atrial standstill has been attributed in part to the digenic inheritance of polymorphisms in the atrial-specific gap junction connexin 40 (Cx40) in conjunction with an *SCN5A* mutation.

METHODS: Family members of an apparently sporadic case of atrial standstill underwent genetic screening of *SCN5A* and atrial-specific genes including Cx40. Biophysical properties of the wild type (WT) and the mutant *SCN5A* channels in a heterologous expression system were studied using the whole-cell patch clamp technique.

RESULTS: A novel *SCN5A* mutation, L212P, was identified in the proband (11 years old) and his father. The father was in normal sinus rhythm while the proband had no P waves in his surface ECG and his right atrium could not be captured by pacing. The recombinant L212P Na channel showed a large hyperpolarizing shift in both the voltage-dependence of activation (WT: -48.1±0.9 mV, L212P: -63.5±1.5 mV, $p<0.001$) and inactivation (WT: -86.6±0.9 mV, L212P: -95.6±0.8 mV, $p<0.001$), and delayed recovery from inactivation. Further screenings for genetic variations that might mitigate L212P dysfunction revealed that the proband, but not his father, carries Cx40 polymorphisms inherited from his asymptomatic mother.

CONCLUSIONS: These results suggest that genetic defects in *SCN5A* most likely underlie atrial standstill and the coinheritance of Cx40 polymorphisms may be a possible genetic factor that modifies the clinical manifestation of this inherited arrhythmia.

Key words: Atrial standstill, *SCN5A*, connexin 40, incomplete penetrance, polymorphism,

SNP, sudden death

List of abbreviations used in the manuscript:

Cx40: Connexin 40, WT: wild-type, AS: atrial standstill, LQT3: type 3 long QT

syndrome, SNP: single nucleotide polymorphism, hβ1: human Na channel β1

subunit, SSS: sick sinus syndrome, SA: sinoatrial, TTX: tetrodotoxin

INTRODUCTION

Atrial standstill (AS) is a rare arrhythmia characterized by bradycardia, absence of P waves, and junctional escape rhythm. Several distinct electrophysiological abnormalities have been recognized in AS, with both persistent and transient forms, and total and partial forms [1]. Familial AS without underlying clinical disorders is extremely rare, and AS episodes often appear to be sporadic, which has hampered identification of responsible genes and the pathophysiology underlying the atria-specific nature of this arrhythmia.

Two mutations have been associated with AS so far in the gene encoding the cardiac Na channel α subunit *SCN5A*, which is responsible for dominant disorders of inherited arrhythmias including type 3 long QT syndrome (LQT3) [2], Brugada syndrome [3], and cardiac conduction system disease [4]. We previously identified a missense mutation R367H in a transient AS case complicated with Brugada syndrome [5], and the recombinant channel showed total loss of Na current when expressed heterologously. In contrast, another *SCN5A* mutation, D1275N, found in a small Dutch kindred with progressive AS, showed relatively benign biophysical abnormalities [6]. Moreover, some mutation carriers in this pedigree exhibit a normal electrocardiogram, and further screening of atria-specific genes has demonstrated that the affected individuals exclusively carry both D1275N mutation and homozygous polymorphisms in the regulatory region of connexin 40 (Cx40), supporting an argument favoring digenic inheritance. Cx40 is a gap junction protein predominantly expressed in the atrium and the specialized conduction system, and the single nucleotide polymorphisms (SNPs) result in reduced Cx40 expression *in vitro* [6]. Furthermore, these SNPs have been clinically associated with enhanced atrial vulnerability and increased risk of atrial fibrillation [7]. These results suggest that the D1275N allele alone may not be sufficient to manifest a

severe clinical phenotype in AS, and the Cx40 SNPs may cause a genetic predisposition which in turn disrupts the spatial distribution of Cx40 influencing atrial conduction and excitability.

Here we present an AS family with a novel *SCN5A* mutation, L212P. The proband showed progressive atrial dysfunction leading to atrial standstill. In contrast to the relatively benign biophysical abnormalities in D1275N, L212P showed severe dysfunction when expressed heterologously. In addition to L212P, the proband carries the maternal Cx40 SNPs allele in the regulatory regions. In contrast, the proband's father, who carries the L212P allele but not Cx40 SNPs, is phenotypically normal. Genetic defects in the *SCN5A* allele most likely underlie the arrhythmic substrate, but the coinherited Cx40 SNPs may confer a severe and atria-specific clinical phenotype in this AS pedigree.

METHODS

Patients

A 3-year-old Japanese boy was admitted to a local hospital because of severe bradycardia. He had never had syncope or dizziness, but he was diagnosed as sick sinus syndrome with paroxysmal atrial fibrillation by Holter ECG recordings. A histological specimen from the right ventricular endocardium performed at the age of 5 was normal. At the age of 10, AS with 6.65 sec of the maximum RR interval was evident, and he has been treated with cilostazol 100 mg/day to prevent severe bradycardia [8]. He was admitted to the Hokkaido University Hospital for further examination including DNA diagnostics. Two siblings of the proband's paternal grandfather had died suddenly in their first decade of life, but the rest of

the family members are asymptomatic and phenotypically normal, and no one has received

cardiac pacemaker implantation or had a history of cardiac surgery.

Molecular genetics

Informed consent was obtained from study participants according to the guidelines of the

medical ethics committee of Hokkaido University. Genomic DNA was extracted from

peripheral blood leukocytes by a standard method. PCR was used to amplify the coding

region and flaking intron sequence of *SCN5A*, as previously described [9]. Exon 1 of Cx40 was

amplified as previously reported [6], and the exon 2 that covers the entire coding region of

Cx40 was amplified by using a primer set (Cx40-F2:

5'-TGGAATCCCAGAACATGATAGA-3', and Cx40-R2:

5'-TCAGTTCAGAAGGGAACAGTCT-3') using LA Taq polymerase (Takara Bio). HCN4

gene was amplified as previously described [10]. Amplified PCR products were analyzed by

direct sequencing using an ABI Prism 310 genetic analyzer (Applied Biosystems).

Genotype and allelic frequencies of the Cx40 SNPs in general Japanese population were

determined by TaqMan technology using genomic DNAs of 99 healthy unrelated individuals.

For the SNP at nucleotide -44 (SNP1), a 181 bp fragment of the Cx40 Exon 1 was amplified

from genomic DNA using a primer set (SNP1-F:

5'-AGCAAAGACTCTAAGAGCACAAAAGAG-3' and SNP1-R:

5'-TTCTCCAACTGCCCACGC-3'). And fluorescent probes were designed to target WT

allele (5'-VIC-TATCTGTCGCCTTCC-Q-3') and SNP1 allele

(5'-FAM-TATCTGTCGCTTTCC-Q-3'). Similarly, a 170 bp fragment was amplified for the

SNP at nucleotide +77 (SNP2) using a primer set (SNP2-F:

5'-GCGTGGGCAGTTGGAGAA-3' and SNP2-R:

5'-TTTTATCCAGCCTAGAATCCAAGCT-3'), and fluorescent probes were designed to

target WT allele (5'-FAM-CCTGGACTTTCCTTC-Q-3') and SNP2 allele

(5'-VIC-CCTGGACTTCCCTTC-Q-3'). Primer and probe design was performed by using

Primer Express software (Applied Biosystems), and sequence homogeneity was confirmed by

comparison to all available sequences on the UCSC database by using BLAST. PCR was

performed in 3μl reaction mixtures containing 1.5 μl of TaqMan universal PCR master mix

(Applied Biosystems), 450 nM concentrations of each primer, 100 nM WT probe, 100 nM

SNP probe, and 3 ng of sample DNA. Thermal cycling was performed on the GeneAmp PCR

System 9700 (Applied Biosystems) consisted of 2 min at 50°C and 10 min at 95°C for

AmpliTaq Gold activation, followed by 40 cycles of 92°C for 15 sec and 58°C (for SNP1) or

60°C (for SNP2) for 1 min. Fluorescence intensity of each well of the plate was read by

Prism 7900HT (Applied Biosystems) and the data were analyzed by the SDS 2.0 allele

calling software (Applied Biosystems).

Site-directed mutagenesis

Wild type (WT) human heart sodium channel α subunit (hNav1.5) cDNA was subcloned

into the pCDNA3.1 plasmid (Invitrogen). Site-directed mutagenesis was performed using

Quick-Change site-directed mutagenesis kit (Stratagene). Correct assembly of the mutant was

verified by restriction analysis and the mutated regions generated by PCR were sequenced

completely to identify clones without polymerase errors. The human cell line tsA-201 was

transiently transfected with either WT or L212P plasmid using standard calcium phosphate

method in combination with a bicistronic plasmid (pCD8-IRES-hβ_1) encoding CD8 and the

human Na channel β_1 subunit (hβ_1) to visually identify cells expressing heterologous hβ_1

with Dynabeads M-450 CD8 (Dynal)[11]. Electrophysiological measurements were performed

24-72 hours after transfection. Functional expression studies were performed on multiple

independent recombinants.

Electrophysiology and data analysis

Na currents were recorded using the whole-cell patch clamp technique. Electrode

resistance ranged from 0.8 to 1.5 MΩ. Data acquisition was carried out using an Axopatch

200B patch clamp amplifier and pCLAMP8 software (Axon Instruments). Currents were

filtered at 5 kHz (-3dB, four-pole Bessel filter) and digitized using an analog-to-digital

interface (Digidata 1322A, Axon Instruments). Experiments were carried out at room

temperature (20-22ºC). Capacitance and series resistance were adjusted (75-85%) to obtain

minimal contribution of the capacitive transients. The holding potential was -120 mV. The

bath solution contained (in mM): 145 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES; and 10

glucose, pH 7.35 (adjusted with NaOH). The pipette solution (intracellular solution)

contained (in mM): 10 NaF, 110 CsF, 20 CsCl, 10 EGTA, and 10 HEPES, pH 7.35 (adjusted

with CsOH).

The data were analyzed by using Clampfit (Axon Instruments) and SigmaPlot (SPSS

Inc.). Curve fitting was made using a non-linear least squares minimization method. The time

course of inactivation was fit with a two exponential function: $I/I_{max} = A_0 + A_f \times exp(–t/\tau_f) +$

$A_s \times exp(–t/\tau_s)$, where A_0 is a constant value, A_f and A_s are the fractions of fast and slow

inactivating components, τ_f and τ_s are the time constants of fast and slow inactivating

components, respectively. Steady-state availability for inactivation and conductance-voltage

relationship were measured with standard pulse protocols as shown in the inset. Curves were

fit with the Boltzmann equation, $I/I_{max} = [1 + exp((V - V_{1/2})/k)]^{-1}$ to determine the membrane

potential for half maximal inactivation or activation ($V_{1/2}$) and the slope factor *k*. Data for WT

and L212P are shown with open circles and closed squares, respectively. Recovery from

inactivation were measured by a double pulse protocol shown in the inset and was analyzed

by fitting data to two exponentials: $I/I_{max} = A_f \times [1-\exp(-t/\tau_f)] + A_s \times [1-\exp(-t/\tau_s)]$.

Results are presented as means ± standard error, and statistical comparisons were made using the unpaired Student's *t*-test. Statistical significance was assumed for $p < 0.05$.

RESULTS

1. Clinical characteristics

The proband was diagnosed as sick sinus syndrome complicated with paroxysmal atrial fibrillation at the age of 3. Atrial electrical activity partially remained at this time, but was progressively disrupted until the time that total atrial inexcitability was evident at the age of 11. A permanent pacemaker was not implanted because he remains asymptomatic, and cilostazol was effective in preventing severe bradycardia. The ECG on admission showed the absence of P waves and a junctional rhythm (HR=40 bpm) with ventricular escape beats (Fig 1A). The duration of the QRS complex and QTc interval were normal. A Holter ECG recording after terminating cilostazol exhibited a junctional rhythm with a maximum RR interval of 5.42 sec (Fig 1B). Organic heart diseases were excluded by chest X-ray, echocardiography, and magnetic resonance imaging. Intracardiac recordings performed in the absence of drugs by standard procedures showed a junctional rhythm with ventricular escape beats (Fig 1C). No atrial activity was observed and the His-ventricular interval was slightly prolonged (56 ms). AS was verified because of the absence of electrical activity in the atrial electrograms or a P wave during pacing at the maximum output of the stimulator (Fig 1D). Low frequency deflections in the His bundle recordings preceding the His deflections in Fig 1C and 1D may represent the residual activities at which atrial capture cannot be achieved. Neither programmed electrical stimulation for ventricular arrhythmias nor provocation test

using class IC drugs was performed.

2. Genetic screening

Genetic screening was performed on three cardiac ion channel genes that have been previously associated with AS, atrial fibrillation, or sick sinus syndrome: the cardiac Na channel *SCN5A*[6,12], a component of I_f current (hyperpolarization-activated cyclic nucleotide gated, or HCN4) [10], and Cx40 [6]. No mutation was found in HCN4, or in the coding region of Cx40 (exon 2). A T-to-C transition was found at the exon 6 of *SCN5A* leading to amino acid substitution of proline for leucine 212 (L212P, Fig 2A) located at the extracellular loop connecting the transmembrane segments 3 and 4 of domain 1 of the cardiac Na channel, Nav1.5 (Fig 2B). This sequence variation was not found in 400 normal chromosomes, consistent with a disease-related mutation. Genomic sequencing of family members showed that the father is a silent L212P mutation carrier (Fig 2C). No arrhythmia has been documented in the paternal grandfather who died of gastric cancer; however, his two brothers died suddenly during the first decade of life. It is possible that these two individuals were also the L212P carriers and developed lethal arrhythmias. A 12-lead ECG of the proband's father showed normal sinus rhythm with non-specific ST depression in the inferior leads (Fig 2D). Genetic screening of the entire coding region of *SCN5A* revealed no other mutations or polymorphisms except for a synonymous SNP (D1819D, heterozygous) in the proband and his father. ECGs of the proband's mother and maternal grandmother were normal.

The genotype of the Cx40 SNPs was determined using genomic DNA of healthy unrelated Japanese individuals. Two Cx40 SNPs in the promoter region and exon 1 showed complete linkage disequilibrium: all subjects with allele G at position -44 had allele A at +71,

and vice versa. The genotype frequencies were, common: -44GG/+71AA (62/99, 62.6%), heterozygous: -44GA/+71AG (32/99, 32.3%), and homozygous SNPs: -44AA/+71GG (5/99; 5.1%). The allelic frequencies of wild type and polymorphisms of Cx40 were 78.8% and 21.2%, respectively. These values were nearly identical to the ones previously reported [7], suggesting minimal ethnic variability in the genetic frequencies of these SNPs. Heterozygous SNPs were found in the proband, his mother and maternal grandmother. The proband's father showed a common genotype (Fig 2C). Homozygous SNPs were not found in the family.

3. Functional characterization of the L212P mutant Na channel

Fig 3A illustrates representative whole-cell current traces from cells expressing WT or L212P Na channels in the presence of co-expressed hβ_1. L212P showed a robust current with a peak current density comparable to WT. The macroscopic current decay fit with a biexponential function showed that the time constants and the fractions of slow and fast components were comparable. The persistent Na current, a characteristic of most mutant Na channels responsible for LQT3 [13], was not evident in cells expressing L212P. The voltage-dependence of steady-state inactivation of L212P showed a significant hyperpolarizing shift by an average of -9 mV ($V_{1/2}$: WT= -86.6±0.9 mV, n=17; L212P: -95.6±0.8 mV, n=11; p<0.001) (Fig 3B). Similarly, the voltage-dependence of activation of L212P showed a large negative shift by an average of -15 mV ($V_{1/2}$: WT= -48.1±0.9 mV; n=19, L212P: -63.5±1.5 mV; n=11 p<0.001). The slope factor for inactivation was significantly larger in L212P than WT (k_{inact}: WT= 7.22±0.18 mV, L212P=6.62±0.16 mV, p<0.05) but similar for activation (k_{act}: WT= -5.8±0.2 mV, L212P= -5.2±0.4 mV; NS) (Fig 3B). Recovery from inactivation was assessed by a standard double-pulse protocol using a recovery potential of -120 mV. The time constants of fast (τ_f) or slow recovery components (τ_s) were significantly larger in L212P (n=9) than WT (n=11) (τ_f: WT=10.8±2.2 ms;

L212P=20.9±2.0 ms, p<0.001; τ_s: WT=159±31 ms, L212P=253±18 ms, p<0.01), and the

fraction of the slow component (A_s) was significantly larger in L212P (WT: 0.16±0.02;

L212P: 0.58±0.03; p<0.05), indicating that the recovery from inactivation was significantly

slower in L212P (Fig 3C).

DISCUSSION

The cardiac Na channel α subunit, encoded by *SCN5A*, is responsible for the generation

and propagation of action potentials in the atrium, ventricle and specialized conduction

system. Mutations in *SCN5A* have been identified in hereditary ventricular arrhythmias

including LQT3 [2], Brugada syndrome [3], and cardiac conduction system disease [4]. Biophysical

studies of Na channel function have shown that mutations that evoke LQT3 cause excessive

Na current (gain-of-function), delaying repolarization and causing early after depolarizations

resulting in Torsades de Pointes [13], whereas Brugada syndrome mutations reduce Na current

(loss-of-function), causing premature epicardial repolarization and phase 2 reentry leading to

ventricular tachycardia or fibrillation [14]. *SCN5A* mutations have also been identified in

supraventricular arrhythmias such as congenital AV block [15], sick sinus syndrome (SSS) [12],

and AS [5,6], but the mechanisms are less well defined as compared with those of ventricular

arrhythmias. The present study describes clinical, genetic and biophysical features of the

novel *SCN5A* mutation, L212P, found in a family with congenital progressive AS.

The heterologously expressed L212P mutant channel exhibited profound gating

abnormalities in both activation and inactivation. However, primary dysfunction of the

sinoatrial (SA) node due to the *SCN5A* mutation is not the plausible pathogenesis of

congenital AS, because the action potential in the SA node is largely calcium channel

dependent and is not associated with tetrodotoxin (TTX)-sensitive Na current[16], while *SCN5A*

encodes TTX-insensitive Na current. SA node dysfunction due to the failure of the impulses to propagate into the adjacent atrial myocardium, referred to as exit block, is the most likely mechanism for congenital AS. The large hyperpolarizing shift (15 mV) of activation in the L212P channel reduces the threshold potential for activation, leading to increased Na current, and the hyperpolarizing shift (9 mV) of steady-state inactivation and delayed recovery from inactivation reduces Na channel availability and slows cardiac impulse conduction. Consequently, L212P evokes gating effects suggestive of both LQT3 and Brugada syndromes, although the L212P carriers do not exhibit the electrocardiographic features of these syndromes. Such opposing gating properties have been previously shown in an *SCN5A* mutation, G514C, responsible for isolated cardiac conduction disease [17]. The G514C channel exhibits a depolarizing shift in both activation and inactivation. It is assumed that the balance of the opposing gating abnormalities as demonstrated in G514C, under the influence of additional factors involved in impulse propagation, may elicit exit block in the SA node. This exit block may progress to total AS in the L212P carrier without evoking electrophysiological manifestations of Brugada syndrome or LQT3. Furthermore, biophysical abnormalities of L212P are more severe than those of D1275N which showed small hyperpolarizing shift (-3.6 mV) in activation only in the absence of co-expressed β1 subunit[6]. Functional severity of the Na channel mutation may be, at least in part, attributable to the fact that the symptomatic individuals in the Dutch family exclusively carry both D1275N and the homozygous Cx40 SNPs, whereas the proband of our case exhibited AS with L212P and heterozygous Cx40 SNPs.

Dissociation between genotype and phenotype is occasionally observed in inherited diseases; mutation carriers could exhibit a normal phenotype and remain asymptomatic. This phenomenon, referred to as incomplete penetrance, has been well characterized in congenital

LQTS [18]. The current study and recent reports clearly show that incomplete penetrance is commonly observed in all three AS pedigrees reported so far [5,6], and it may account for the fact that familial AS is extremely rare and AS often appears to be sporadic. Furthermore, these observations suggest that *SCN5A* mutations alone are not sufficient to confer a severe AS phenotype; the clinical consequence of the mutations is determined in individual-specific or family branch-specific manners, rather than a mutation-specific manner, probably influenced by some environmental factors or genetic modifiers associated with atrial conduction. Several lines of evidence implicate the genetic variations of *SCN5A* or Cx40 accompanying the *SCN5A* mutations. Benson *et al.* reported six heterozygous compound *SCN5A* mutations in three congenital SSS families with recessive inheritance [12]. Affected individuals of one pedigree, who exhibit progressive atrial standstill, exclusively carried heterozygous compound *SCN5A* mutations of both G1408R and P1298L. Family members carrying only one of these alleles showed a normal ECG or only first-degree atrioventricular conduction block, despite the fact that G1408R results in a non-functional channel. These observations suggest that a single *SCN5A* mutant allele, even a non-functional allele, may not be sufficient to confer a severe clinical phenotype, and an additional *SCN5A* mutation on the other allele may have a significant impact on the electrophysiological manifestations.

Action potential in cardiac tissue is greatly affected by both membrane excitability due to Na channel availability and gap junction coupling. Cardiac Na channel Nav1.5 is localized in the intercalated disks between neighboring myocytes[19], and interplay between these molecules has significant relevance under pathological conditions in which one pathological change may compensate for another [20]. Cx40 is a gap junction protein predominantly expressed in the atrium and specialized conduction system, and Cx40-deficient mice showed significantly prolonged P wave duration, PQ interval, and QRS duration [21]. Interplay between

the Na channel and Cx40 is also suggested in heterozygous *SCN5A* knockout mice. These mice show progressive cardiac conduction disturbances with age and myocardial fibrosis, and exhibit a significant decrease in Cx40 expression in the atria, together with P wave prolongation [22]. In the Dutch AS pedigree, affected individuals exclusively carry both the *SCN5A* mutation (D1275N) and homozygous SNPs in the regulatory region of Cx40 which reduces Cx40 expression *in vitro* [6]. Furthermore, these CX40 SNPs have been clinically associated with enhanced atrial vulnerability and increased risk of atrial fibrillation [7]. The prevalence of Cx40 SNPs in the general population is not rare and the Cx40 promoter activity of homozygous SNP is less than 50% of WT [6], suggesting that the heterozygous Cx40 gives rise to intermediate promoter activity, and the clinical manifestation of Cx40 SNPs alone may not be apparent. Nevertheless, the likelihood of the chance occurrence of Cx40 SNPs and *SCN5A* mutation is rare, and it may explain the phenotypic difference between two L212P carriers in the AS family, and the extremely low prevalence of this arrhythmia. We believe that genetic defects in *SCN5A* most likely underlie AS, but additional genetic factors that modulate atrial electrical coupling are relevant to the clinical manifestation of this inherited arrhythmia. Further genetic and functional studies are required to confirm this mechanism.

Study limitation

The size of the family in the present study was quite small and clinical or genetic information was available from only several members in this pedigree. Extensive efforts to gather more genomic and electrophysiological data in AS patients are required to determine the relevance of *SCN5A* and Cx40 polymorphisms in this rare inherited arrhythmia. Experiments using genetically engineered animals or mathematical models are required to elucidate the direct effects of Cx40 polymorphisms on Na channel function.

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FIGURE LEGENDS

Fig 1. Electrocardiographic findings of the proband

A. Twelve-lead ECG of the proband. Tracings of leads I-III and V1 are shown. Junctional rhythm (HR 40) with a ventricular escape (second QRS). Note the absence of P waves.

B. Holter ECG recording after terminating cilostazol. AS with a maximum RR interval of 5.42 sec was documented during sleep.

C. Tracings of intracardiac recordings. Junctional rhythm and ventricular escape beats (second and fourth QRS). HRA, representing high right atrium, shows far-field V signals but not atrial activities. Low frequency deflections in the His bundle electrogram (HIS) preceding the His deflection may represent the residual atrial activities although there are no areas in the right atrium that was captured that by pacing with maximum output (D). The His-ventricular interval was slightly prolonged (56 ms). RVA represents right ventricular apex.

D. Atrial pacing with the maximum output of the stimulator (9.9 V, 1.0 ms) failed to capture the atria. STIM represents atrial pacing artifacts.

Fig 2. Results of DNA sequencing, location of the mutation, and the pedigree

A. Sequence electropherogram for exon 6 of *SCN5A* of the proband. The arrow indicates heterozygous missense mutation of proline (CCG) for leucine-212 (CTG).

B. Predicted topology of the cardiac Na channel Nav1.5 is illustrated with the location of three mutations associated with AS: L212P (this report), R367H [5], and D1275N [6]. Transmembrane segments S1-S6 in each domain are shown with numbers and boxes.

C. Pedigree of the AS family. Open symbols indicate unaffected or undetermined

individuals. Closed and shaded symbols indicate genetically affected individuals

with or without symptoms, respectively. The genotypes below each person's symbol

are, from top to bottom, as follows: *SCN5A*-L212P status (WT and MT indicate wild

type and heterozygous L212P, respectively), Cx40 -44 bp SNP (common GG, and

heterozygous GA), and Cx40 +71 bp SNP (common AA and heterozygous AG). The

proband has inherited the L212P allele from his father and the Cx40 SNPs allele

from his mother. SCD represents sudden cardiac death.

D. Twelve-lead ECG of the proband's father (asymptomatic mutation carrier) shows

normal sinus rhythm. None-specific ST depression was evident in the inferior leads.

Fig 3. Biophysical properties of L212P mutant Na channel

A. Representative whole-cell current traces of WT and L212P Na channels expressed in

tsA-201 cells in the presence of co-expressed human β1 subunit. Currents were

elicited by 20 ms test pulses (from -90 to +80 mV, 10 mV step) form the holding

potential of -120 mV. Currents of the representative traces were normalized with the

peak currents to illustrate the time course of current decay of each channel. Scale

bars indicate 1 nA and 5 ms.

B. Steady-state availability for inactivation and conductance-voltage relationship was

measured with standard pulse protocols as shown in the inset. Normalized peak

current and conductance were fit with the Boltzmann equation (see Methods). Both

inactivation and activation curves were significantly shifted in the negative direction

in L212P (n=17) compared to WT (n=11).

C. Recovery from inactivation was measured by the standard double pulse protocol as

depicted in the inset. Fractional recovery was fit with the double exponential

equation (see Methods). Recovery was significantly slower in L212P (n=9) than WT

(n=11).

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A

AACT G AAT TT GT G GAC CN GGG CAAT GT CT CAG CC TT AC GCA

Leu (CTG) →Pro (CCG)

B

L212P
R367H
D1275N

C

SCD SCD

WT
GA
AG

MT
GG
AA

WT
GA
AG

SCN5A
Cx40 -44 SNP
Cx40 +71 SNP

Proband

MT
GA
AG

D

I
II
III
aVR
aVL
aVF

V1
V2
V3
V4
V5
V6