Molecular and Clinical Characterization of a Novel SCN5A Mutation Associated with Atrioventricular Block and Dilated Cardiomyopathy

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Ge: SCN5A Mutation Causing AVB and DCM

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**Background**—Increased susceptibility to dilated cardiomyopathy (DCM) has been observed in patients carrying mutations in the *SCN5A* gene, but the underlying mechanism remains unclear. In this study we identified and characterized, both in vitro and clinically, an *SCN5A* mutation associated with familial progressive atrioventricular block (AVB) of adult onset and DCM in a Chinese family.

**Methods and Results**—Among 32 family members 5 were initially diagnosed with AVB after age 30; 4 were studied, 3 of whom later developed DCM. We found a heterozygous single-nucleotide mutation resulting in an amino acid substitution (A1180V) in all studied patients and in 6 other younger unaffected members, but not in 200 control chromosomes. When expressed with the β1 subunit, the mutated channels exhibited a −4.5 mV shift of inactivation with slower recovery leading to a rate-dependent Na⁺ current reduction, and a moderate increase in late Na⁺ current. Clinical study revealed that while QRS duration decreased with increasing heart rate in non-carrier family members, this change was blunted in unaffected carriers whose electrocardiograms and heart function were normal. Resting QTc interval of unaffected carriers was significantly longer than that of non-carriers, even though it was still within the normal range.

**Conclusions**—A1180V expresses a mild Na⁺ channel phenotype in vitro and a corresponding clinical phenotype in unaffected mutation carriers, implying that A1180V caused structural heart disease in affected carriers by disturbing Na⁺ influx, and hence, cellular Na⁺ homeostasis. The high penetrance of A1180V suggests this phenotype as a high risk factor for DCM with preceding AVB.

**Key words**—dilated cardiomyopathy, late current, mutation, rate-dependence, sodium channel
Introduction

Dilated cardiomyopathy (DCM) is the most common form of heart muscle disease and a leading cause of congestive heart failure.\(^1\) In a large percentage of cases, DCM is idiopathic and its underlying cellular and molecular bases remain poorly understood. Idiopathic DCM affects 40 out of every 100,000 people.\(^2\) About \(~30\%\) of cases are familial,\(^3\) which indicates that genetics may play a role in these cases. Genotypes that are known to cause familial DCM include mutations of genes encoding proteins of the cytoskeleton and contractile apparatus, Ca\(^{2+}\)-handling proteins, and the lamin A/C protein.\(^4\) Recent evidence\(^5-7\) also implicates mutations of genes encoding cardiac ion channels, such as \(SCN5A\), in familial DCM.

\(SCN5A\) encodes the \(\alpha\)-subunit, Nav1.5, of the cardiac Na\(^+\) channel that is responsible for the rapid depolarization of action potentials in cardiomyocytes. Previously, \(SCN5A\) mutations were identified mainly as a genetic risk factor for rhythmic disorders of the heart, such as long QT syndrome, Brugada syndrome, isolated cardiac conduction disease, and other forms of arrhythmia, that were attributed to electrical abnormalities without myocardial damage. However, recent data suggest a possible pathogenic role of cardiac Na\(^+\) channel defects in the development of myocardial structural abnormalities. An early study of a set of familial DCM cases narrowed the genes responsible for the diseases to chromosome 3p22-p25, which contains \(SCN5A\).\(^8\) Case reports describing patients with \(SCN5A\) mutations and DCM (in a white family carrying a single nucleotide polymorphism, SNP, S1103Y\(^9\)) or degenerative changes in the conduction system\(^10\) also exist. After refining the genetic mapping of the DCM locus on chromosome 3p22-p25, Olson et al.\(^7\) determined that \(SCN5A\) was a candidate gene for DCM; correlation analysis further
suggested that five SCN5A mutations—T220I, R814W, F851Xfs, D1275N, and D1595H—increase susceptibility to early-onset DCM and atrial fibrillation (AF). McNair et al.\(^6\) independently reported that D1275N segregated with DCM with a conduction defect. Frustaci et al.’s\(^1\) pathological study showed evidence of myocardial damage in patients with Brugada syndrome and SCN5A mutations. Most recently, a new homozygous mutation, R814Q, was reported\(^1\) to be associated with Brugada syndrome and right ventricular structural abnormalities.

Although not all SCN5A mutations that are linked to myocardial damage and structural abnormalities have been functionally studied in vitro, those that have been studied generally are characterized by reduced Na\(^+\) currents, or a phenotype known as loss of function. Evidence for SCN5A mutations causing myocardial damage and DCM also is emerging from experimental studies, despite some limitations.\(^7,\)^\(^1\) In mice, targeted disruption of an Scn5a allele reduces gene expression by 50\%,\(^1\) which is accompanied by an age-related cardiac fibrosis and gap junction disarrangement that causes conduction impairment.\(^1\) Ectopic expression of the zinc finger protein Snail in transgenic mice results in Na\(^+\) channel down-regulation and a phenotype combining progressive DCM and a conduction defect.\(^1\) Nevertheless, many loss-of-function SCN5A mutations are not linked, or have not yet been linked, to structural disease. Therefore, it remains difficult to simply correlate a loss-of-function phenotype to structural disease.\(^6,\)^\(^7,\)^\(^1\)

Currently, little information is available on how and why loss of function due to SCN5A mutation increases a person’s susceptibility to myocardial damage and structural disease.

Herein, we identified and studied a novel SCN5A mutation, A1180V, in a three-generation Chinese family. Some family members carrying A1180V suffer from DCM that was preceded by
progressive atrioventricular block (AVB). We conducted cellular electrophysiological experiments to determine the biophysical properties of the mutant Na⁺ channel. We also examined ECG, cardiac function, and response to exercise in family members who carry the mutation but have not yet developed the disease and compared them with non-carrier family members. We hope that systematic characterization of the A1180V phenotype both in vitro and clinically will help elucidate whether and how this mutation causes structural disease of the heart.

Methods

Clinical examination—We obtained written informed consent from all participants, and the study was conducted in compliance with the guidelines for genetic research using protocols approved by the Ethics Committee of Zhongshan Hospital, Fudan University. The proband (II-1 in Table 1) in the pedigree and his first-degree relatives underwent clinical evaluation, including medical and family history review, physical examination, neurological examination, 12-lead ECG, and echocardiography examinations. During the course of the study, other family members were invited to undergo similar clinical evaluation.

Exercise testing—Asymptomatic family members participated in exercise testing on a voluntary basis. All participants performed the Bruce protocol on the treadmill under ECG and blood pressure monitoring; their 12-lead ECG and echocardiograms were recorded at rest and immediately after exercise when the heart rate reached >150 bpm. A two-dimensional
Echocardiogram was taken by an echocardiographic machine (Siemens Acuson Sequoia Ecompass III, Siemens, Germany) using the standard parasternal and apical approaches with the individual in the left lateral decubitus position. We manually measured left ventricular end-diastolic and end-systolic dimensions and confirmed the values by automated measurement through a program (Axius Auto EF) installed in the echocardiographic machine. Doppler echocardiography was performed at the same time to assist in the estimation of cardiac output. ECG parameters were measured manually and independently by two participating investigators. Each parameter is an average of at least three successive heart beats.

Molecular genetic analysis—Genomic DNA was isolated from the patients’ blood. Each of the coding exons (except exon 28) of SCN5A and their corresponding exon–intron boundaries, was amplified by PCR from the genomic DNA. Exon 28, due to its large size, was amplified in three overlapping fragments. Supplemental Table I lists all PCR primers used. The resulting PCR product was sequenced using an ABI377 automated sequencer (Applied Biosystems, Foster, CA). Ambiguous bases in, or within 10 bases outside of, an exon were either re-sequenced or sequenced in the opposite direction. The protocol was repeated for all coding exons of SCN5A in each individual under examination. We also analyzed exon 20 from the chromosomes of 200 unrelated healthy Chinese individuals.

Mutagenesis and heterologous expression—The SCN5A coding sequence has several major polymorphic variations. After analyzing polymorphic patterns of the family members whose
SCN5A gene was sequenced (n=23), we chose an SCN5A cDNA clone\textsuperscript{17} that most closely matched the genes of both the patients and unaffected family members (Supplemental Table II); we then used it as the wild-type gene in the functional study. In addition to genomic variations, Nav1.5 channels are also present in two splice variants, Q1077 and Q1077del.\textsuperscript{17} The cDNA clone used in this study encodes the full-length, 2,016 amino acid channel that includes Q1077. A mutated cDNA containing A1180V was constructed based on this background using PCR-based site-directed mutagenesis, and the mutation was confirmed by sequencing. The cDNA were transiently transfected using an Effectene\textsuperscript{TM} reagent (QIAGEN, Germany) into HEK293 cells stably expressing the β1-subunit of the human Na\textsuperscript{+} channel. The cells were also co-transfected with cDNA of a green fluorescent protein that served as an expression indicator. The wild-type SCN5A cDNA and HEK293 cells stably expressing the β1-subunit were kindly provided by Dr. Makielski at the University of Wisconsin.

**Cellular electrophysiology and protein expression analysis**—Experiments were performed 48–64 hours after transfection. We conducted patch-clamp recordings at room temperature (22°C) at a holding potential of −120 mV (if not otherwise indicated). The extracellular solution contained (in mM): NaCl 140, KCl 4, CaCl\textsubscript{2} 1.8, MgCl\textsubscript{2} 0.75, and Hepes 5, at pH 7.4. The intracellular solution contained (in mM): CsF 120, CsCl 20, EGTA 5, and Hepes 5, at pH 7.2. We calculated voltage dependencies of activation and steady-state inactivation using a Boltzmann function, and quantified time courses of inactivation and recovery from inactivation by fitting measured data with a two-exponential function.
Expression level of Nav1.5 channel protein was assessed by a standard SDS-PAGE/Western blotting protocol using an antibody against human Nav1.5 (Alomone Labs, Jerusalem, Israel).

Statistical analysis—Statistical data are reported as mean±S.E. Statistical differences between wilt-type (or non-carrier) and mutation (or carrier) groups were tested using Student’s t-test without adjustment for multiple comparisons. If the normality test or equal variance test failed, the nonparametric Mann-Whitney rank sum test was used for group comparison. In cases where sample sizes were small, an additional nonparametric permutation t-test (3000 permutations) also was applied to confirm the statistical test results. Statistical significance was determined at \( P<0.05 \).

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Clinical characteristics of the patients—In 1987, the then 37 year-old proband (II-1 in Table 1) was referred to the authors because of palpitation and first-degree AVB. Four years later, the proband developed complete AVB, AF, and cardiac dilation. A pacemaker (VVI) was implanted in 1997, but cardiac dysfunction continued and this patient died of severe congestive heart failure in 2005. During the proband’s clinical visits, we were informed that his relatives had similar
symptoms. This is a family in which most adult members engaged in farm work, with the exception of some younger members. The proband’s father (I-1) developed complete AVB and AF at age 47 and died 6 years later; unfortunately his clinical records were not available to us and there was no record of his cardiac functions and conditions, although the proband’s father's sister (I-3) lived into her seventies with no history of heart disease. The proband’s grandmother died suddenly at the age of 52; her clinical records were not available either. Therefore, we examined other members of the pedigree and continued to follow-up. By 2005, three members of the pedigree in addition to the proband had been diagnosed as affected patients (II-3, II-7, and III-1); their initial clinical manifestation was AVB, with an age of onset ranging from 31 to 37. Within 5 years after onset, II-3 and II-7 developed left ventricular dilation and dysfunction, as revealed by echocardiography, with a left ventricular diameter >60 mm and a left ventricular ejection fraction <40%. II-3 died of severe congestive heart failure in 2006. III-1 was diagnosed with first-degree AVB at the age of 31, but as of 2006 no evidence of cardiac dilation has appeared. A younger female member (III-13, age 23) had a borderline long PR interval (202 ms) seen on resting ECG, but she was otherwise asymptomatic. All patients diagnosed as having dilated cardiomyopathy and systolic dysfunction were treated with heart failure medications. Figure 1 displays examples of ECG recordings and echocardiograms of affected patients, and Table 1 summarizes the clinical presentation of the pedigree. None of the patients in the pedigree showed any sign of long QT or Brugada syndromes, and none had myopathy, other neuromuscular disease, or neuronal disease.
Molecular genetic findings—By sequencing the entire coding region and exon–intron boundaries of the proband’s SCN5A gene, we identified a heterozygous mutation, c.3539C>T, located in exon 20 (Fig. 2A). This mutation causes an amino acid substitution, A1180V, that is predicted to be in the intracellular linker between DII and DIII of the Nav1.5 channel, near the ankyrin-G binding sequence. Figure 2B depicts the approximate location of A1180V and its relation to other known DCM-causing SCN5A mutations. A1180V was detected in all four affected members and in six younger unaffected members of the pedigree, whereas it was absent in the rest of the unaffected family members. The mutation was not detected in chromosomes of 200 healthy unrelated subjects. The lower panel of Figure 2A gives an example of the sequencing results, and Figure 2C displays the distribution of A1180V across the affected family.

In addition to A1180V, we also detected four single nucleotide variants (Supplemental Table II) that differed from their counterparts in a reference sequence (GenBank AC137587) in the entire coding region of SCN5A. These were the only coding variants found in the family members examined. Three variants—c.87G>A (synonymous), c.3578G>A (R1193Q), and c.5457T>C (synonymous)—were reported previously in healthy Chinese cohorts, and their allele frequencies in our study were similar to those reported in the literature. The fourth variant, which is non-synonymous and causes an I244T amino acid change, has not been reported previously. However, it was only found in four unaffected individuals who were parent/siblings, and therefore we concluded that it was not related to the disease under study. Haplotype analysis (see Supplemental Figure I) of the variants together with two intronic variants near exon–intron boundaries revealed that: (1) A1180V is linked to the most common haplotype that has major
alleles (allele frequencies >0.4) of healthy subjects at all sites of variation, (2) these alleles precisely match the wild-type cDNA that we used in the following functional study, and (3) no minor variants or minor haplotypes, except A1180V, were associated with the disease.

Results of the cellular electrophysiological study—We characterized the whole-cell currents of the mutated Na⁺ channels (A1180V channels), and compared them with the wild-type Na⁺ channels. A1180V channels and wild-type channels shared the same I–V relationship, had indistinguishable maximal current densities, and expressed at a similar protein level as assessed by Western blotting (Figs. 3A–3C). A1180V did not significantly affect the channel activation, as can be seen from the voltage dependence and time course of activation (Figs. 3D and 4A).

However, A1180V induced a negative shift (−4.5 mV) of the steady-state inactivation curve (Fig. 4A) but had little influence on the time course of current decay (Fig. 3D). In addition, the A1180V channels exhibited a mildly increased late current that was not completely inactivated at the end of a 250-ms depolarizing pulse (Fig. 4B). Recovery from inactivation also differed between the A1180V and wild-type channels. At all voltages (−120, −100, and −80 mV) for which recovery was examined, the A1180V channels recovered more slowly than the wild-type channels (Fig. 5). The slower recovery led to a moderate level of Na⁺ current decrease when the A1180V channels were repetitively activated by a train of depolarizing pulses (Figs. 6A,B). The current loss depended on the rate of the repetitive pulse, being greater at a higher pulse rate (Fig. 6C).
Results of exercise testing—Given the cellular electrophysiological finding that A1180V changes the biophysical properties of Nav1.5 channels, we wondered whether a clinical phenotype consistent with the mutant channel phenotype is present in A1180V carriers. Using ECG and echocardiography, we examined the heart’s electrical properties and hemodynamic function and their response to heart rate increase in the unaffected carriers of the pedigree. We also compared measurements obtained from the unaffected carriers with those from the non-carriers of the family (Fig. 2C). Heart rate increase was achieved through exercise. Due to consideration for the safety and comfort of the participants, we avoided using any invasive procedures or agents to accelerate heart rate. All characteristic parameters of ECG and cardiac function of the unaffected carriers were within the normal ranges (Table 2). When treated as groups, most parameters between the non-carrier group and the carrier group were not statistically different. However, three exceptions exist. First, QRS duration of the unaffected carrier group was shorter. Second, the response of QRS duration to exercise differed between the two groups. For the non-carrier group, the mean QRS duration was shortened by $-7.6\pm1.7\%$ when heart rate increased from $\sim70$ bpm to $\sim150$ bpm; in contrast, the mean QRS duration of the unaffected carrier group was slightly prolonged by $0.6\pm1.5\%$. Figure 7A shows superimposed QRS complexes before and after exercise recorded from an unaffected carrier (III-3) and from a non-carrier (II-13). Figures 7B and 7C statistically summarize the relationship of heart rate and QRS duration as well as the relationship of heart rate and cardiac index of the two groups. Third, the unaffected carrier group demonstrated a statistically longer, though still normal, corrected QT interval (QTc) at rest. After exercise, however, the QTc interval between two groups was no longer statistically different.
Discussion

We have presented experimental data identifying and characterizing A1180V obtained from laboratory research and information collected from 20 years of clinical follow-up and research of the Chinese family. The major findings of the study are as follows: (1) In the affected pedigree, A1180V segregated with DCM that was preceded by progressive AVB with onset in the third decade of life; (2) A1180V channels exhibited a range of mild phenotypes; and (3) Mild phenotypic changes were detected in resting and exercise ECG of unaffected carriers of the pedigree. In the discussion below, we further analyze and interpret these findings to evaluate their implications for the relationship between A1180V and the familial DCM in this pedigree.

To our knowledge, the A1180V mutation has never been reported before. In our study we found it was only present, in heterozygous form, in the proband’s pedigree and never in individuals unrelated to the pedigree. Clinically, carriers in the proband’s generation all manifested DCM with preceding AVB. In contrast, none of the non-carriers in the family have shown any sign of similar heart disease. We did not find segregation of any other mutation, minor variants, or minor haplotype of SCN5A with the disease, nor were they uniquely linked with A1180V. Finally, the disorders associated with A1180V are apparently restricted to the heart. These genetic and clinical findings indicate that A1180V is inherited in an autosomal dominant pattern and has an age-related, high penetrance for familial DCM with preceding AVB. In general, familial DCM has various patterns of inheritance and phenotypic expression that depend on the defective genes.\(^1\)\(^,\)\(^4\) The SCN5A mutations that are thought to be a primary cause of DCM
are characterized by autosomal dominant transmission and an age-related and relatively high penetrance for DCM with various conduction defects and arrhythmias. 6, 7 Similarities in both genetic and phenotypic aspects between A1180V and other known DCM-causing SCN5A mutations prompted us to theorize that A1180V primarily accounts for the initiation of familial DCM and AVB in the present pedigree. However, additional genetic or environmental factors may play a role in causing disease. For example, because the A1180V channel loses current only at high heart rates, it is conceivable that physical activity and lifestyles that increase average daily heart rate may contribute to the onset of the disease.

Although evidence from both patients and animal models has strongly implied that mutation of cardiac Na+ channels can cause myocardial damage and structural abnormality, little is known about the underlying mechanism. What has been reported consistently for all known DCM-causing SCN5A mutations is the loss-of-function phenotype, although there are various molecular mechanisms leading to Na+ current reduction. It has been postulated that Na+ current reduction disturbs cellular Na+ homeostasis, which subsequently can affect cellular pH and Ca2+ homeostasis, thus compromising excitation-contraction coupling and energy production mechanisms. 11 However, alternative hypotheses also exist. For example, evidence exists for direct interaction of cytoskeletal proteins with Nav1.5 channels. 18, 21 Channel mutations may compromise such interaction, disturb the cytoskeletal network, and eventually lead to cell damage. This mechanism, however, does not necessarily involve any change in Na+ current. Distinguishing between these mechanisms requires determining whether an SCN5A mutation alters the channel current.
When examined in vitro, A1180V channels are distinguishable from wild-type channels in two aspects: A1180V channels exhibit a negative shift of steady-state inactivation with a slower recovery that leads to a rate-dependent loss of peak Na\(^+\) current and an increase in the late Na\(^+\) current. Can these changes in Na\(^+\) current cause myocardial damage? Previously, Grant et al.\(^{22}\) reported that ΔK1500 shifted inactivation to a more negative voltage range and induced a large increase in late Na\(^+\) current. The mutation was associated with long QT syndrome, Brugada syndrome, and conduction system disease. Microscopically, ΔK1500 also changed channel activation by inducing a positive shift with a reduced slope factor. A1180V, however, did not affect channel activation. R1193Q, a variant proximate to A1180V, was also observed to cause a negative shift in inactivation with an accelerated current inactivation, and an increase in late Na\(^+\) current.\(^{23}\) This profile is similar to, but not exactly the same, as the A1180V phenotype. However, R1193Q is a common variant present in ~12% of Chinese.\(^{20}\) In our study, heterozygous R1193Q was present in two non-carriers, two unaffected carriers, and one affected carrier (Supplemental Table II). Thus, the pathogenic significance of this variant’s phenotype is unclear.

Remarkably, we found a better match of the A1180V’s phenotype outside of the SCN5A mutations. In rabbit and mouse cardiomyocytes, Wagner et al.\(^{24}\) found that phosphorylation of cardiac Na\(^+\) channels by direct association of the channels with Ca\(^{2+}\)/calmodulin-dependent protein kinase II\(\delta\) \(\text{CaMKII}\delta\) induced a negative shift of steady-state inactivation, slowed recovery from inactivation, and enhanced late Na\(^+\) current. CaMKII\(\delta\) does not affect channel activation and current density, and neither does A1180V. The biophysical profile of cardiac Na\(^+\) channels under the modulation of CaMKII\(\delta\) qualitatively overlaps with the phenotypic profile
of A1180V in all aspects, although quantitatively the effect of CaMKIIδC is more prominent. Transgenic mice overexpressing CaMKIIδC developed DCM and heart failure, and their cardiomyocytes had an abnormally high concentration of cellular Na⁺. Because A1180V and CaMKIIδC share a similar profile in their effect on cardiac Na⁺ channels, we postulate that cellular Na⁺ concentration, and as a consequence, intracellular Ca²⁺ and pH, are altered in a heart that expresses A1180V channels. Unfortunately, because CaMKIIδC also mediates phosphorylation of Ca²⁺ regulatory proteins, at present it is difficult to determine the role of abnormal Na⁺ concentration via Na⁺ channels in the pathogenesis of DCM based on data obtained from CaMKIIδC-transgenic mice.

It needs to be noted that recent studies have shown that the splicing variant Q1077del altered channel phenotype in some SCN5A mutants and common variants. In our functional study, only the Q1077 splicing variant was examined. The phenotype of A1180V on the Q1077del splicing variant background remains to be determined.

Most importantly, we found that the channel phenotype must be present in human carriers if it is to be pathogenic. Our clinical study, as guided by cellular experimental data, revealed a phenotype in the unaffected carriers that can be explained by the channel phenotype. We base this premise on the following analysis. First, the increased level of late Na⁺ current shown in the channel phenotype of A1180V predicted a prolongation of the QT interval. Indeed, we found that the QTc interval of the unaffected carrier group at rest was statistically longer than that of the non-carrier group, even though the difference was small and all individual carriers’ QTc intervals were still normal. The prolongation disappeared with exercise. Such rate-dependent QT
prolongation is typically seen in long QT patients with an SCN5A mutation.\textsuperscript{27} Thus, we conclude that the prolongation of carriers’ QTc duration is substantial, and it is caused by the increased late Na\textsuperscript{+} current. Second, the rate-dependent A1180V channel current reduction predicted a wider QRS duration at high heart rate. In our study, the participants’ heart rate was elevated through exercise. However, it has been well documented that exercise per se shortens QRS duration in healthy human subjects.\textsuperscript{28} In our study, exercise consistently induced QRS shortening in the non-carrier group; interestingly, such shortening was blunted in the unaffected carrier group (Fig. 6). We interpret this relative widening of QRS duration to be a consequence of reduced Na\textsuperscript{+} current at high heart rate. In addition to the rate-dependent change of QRS duration, we noticed that the resting QRS duration of the unaffected carrier group was significantly shorter than that of the non-carrier group, although for individuals their resting QRS durations still fell in the normal range (Table 2). Because women usually have slightly shorter QRS duration than men\textsuperscript{29} we have considered that the difference may be attributed to fewer male participants in the unaffected carrier group than the non-carrier group. However, we further found that a similar difference still exists between the female participants of two groups, suggesting that short QRS duration may also be a part of phenotype of A1180V. A mechanistic explanation for short QRS is not readily available with the data from A1180V channels expressed in HEK293 cells. We speculate that the shorter QRS duration represents an increased Na\textsuperscript{+} current density, possibly due to an increased level of protein expression through an unclear mechanism that may be absent in HEK293 cells but present in the heart. For example, Mohler\textsuperscript{18} reported that disruption of channel ankyrin-G interaction by mutation E1053K blocked Na\textsuperscript{+} expression in cardiomyocytes but not in
HEK293 cells. Further study is needed to explain our observation. Finally, the results of exercise testing confirmed that all unaffected carriers had normal heart function and structure. Therefore, the phenotype shown in the unaffected carriers’ ECG precedes the development of any detectable conduction system defect and heart function abnormality, and most likely it is congenital.

In conclusion, the findings of this study are consistent with the hypothesis that congenital disturbances of cardiac Na⁺ current due to SCN5A mutation can substantially contribute to the development of myocardial damage and, ultimately, to DCM. Thus, these findings strengthen the rationale for future studies in experimental models such as transgenic animals that would allow for more direct tests of the Na⁺ current hypothesis and provide mechanistic insights into the pathogenic link between cardiac Na⁺ channel abnormality and DCM.
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Disclosures

None.
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Figure Legends

Figure 1. Clinical manifestations of progressive AVB and DCM. A. ECG revealing first-degree AVB in patient III-1 at age 32. No sign of cardiac dilation was present at the time when the ECG was recorded. B. ECG showing AF, third-degree AVB, and a slow ventricular escape rhythm with bigeminal ventricular premature beats in patient II-3 at age 53. The patient had cardiac dilation. C. DCM manifests on the echocardiogram as dilated chambers in the proband (II-1) at age 53, 7 years after pacemaker implantation.

Figure 2. Molecular identification of A1180V and its segregation with cardiopathies. A. Results of genomic DNA sequencing analysis contrast carriers and non-carriers. Upper panel: Sequencing data obtained from the proband’s DNA sample showing a heterozygous nucleotide change in exon 20 of SCN5A. The mutation results in an alanine→valine substitution at 1180. Lower panel: Sequencing data from an unrelated control person. B. Location of A1180V in the predicted topologic structure of the Nav1.5 channel. Other mutations previously reported to be associated with DCM are also indicated at their approximate locations. C. Pedigree of the affected family displaying the segregation of A1180V with cardiopathies. Family members are identified by generations and numbers, and male and female members are represented by squares and circles, respectively. Individuals diagnosed with various cardiopathies, including DCM and/or AVB, are marked by darkened symbols, and those deceased are denoted by a slash through the symbol. A symbol containing a dot identifies an individual who had a PR interval of 202 ms at rest. “+/−” indicates individuals who were identified as carriers of the heterozygous
A1180V mutation, and “–/–” indicates non-carriers.

**Figure 3.** Na⁺ currents of the wild-type and A1180V channels. **A.** Representative whole-cell Na⁺ currents elicited by the voltage protocol shown in the inset. **B.** Normalized I–V relationships of peak Na⁺ currents; n=16 and 17 for the wild-type and A1180V channels, respectively. **C.** Expression of the wild-type and A1180V channels. The bar plots compare Na⁺ current densities of the wild-type and A1180V channels. The data were obtained from the maximal peak Na⁺ currents normalized by the capacitance of the cells. The image gives a representative result of Western blotting of cells expressing the wild-type and A1180V channels together with the β1 subunit, using the antibody against the Nav1.5 protein. **D.** Voltage dependencies of activation time and inactivation time constants of the Na⁺ current for the wild-type and A1180V channels. Time to the peak Na⁺ current was taken as the measurement of the activation time. The decay time constants were obtained by fitting a two-exponential function to the decay of the Na⁺ current.

**Figure 4.** Steady-state kinetics of the wild-type and A1180V channels. **A.** Boltzmann distributions of voltage-dependent channel activation and inactivation. The voltage-dependent activation curves were converted from data shown in Fig. 2B; the voltage for half activation and the slope factor for the activation curve of the wild-type channels vs. those of the A1180V channels, respectively, were –35.5±1.4 mV and 5.6±0.4 (n=13) vs. –34.1±1.8 mV and vs. 5.8±0.3 (n=15). Steady-state inactivation was assessed using the protocol shown in the inset. The
voltage for half inactivation and the slope factor for the inactivation curve of the wild-type channels vs. those of the A1180V channels, respectively, were $-78.7 \pm 1.3$ mV and $-5.3 \pm 0.2$ (n=26) vs. $-83.1 \pm 1.6$ mV and $-5.4 \pm 0.3$ (n=31). The difference in the voltage for half inactivation between the wild-type and A1180V channels is statistically significant. B. Comparison of late Na$^+$ currents. The late Na$^+$ currents were measured at the end of 250-ms depolarizing pulses, as shown in the inset. Left panel: Representative recordings of late Na$^+$ currents in response to a depolarization pulse to $-20$ mV. The currents were normalized to the corresponding peak Na$^+$ currents. Right panel: I–V relationships of the late Na$^+$ currents. * indicates significant differences between the wild-type and A1180V channels.

Figure 5. Comparison of recovery from inactivation between the wild-type and A1180V channels. A. Recovery from inactivation at holding potential of $-120$ mV (left), $-100$ mV (center), and $-80$ mV (right). The voltage protocol used to elicit the recovery process is shown in the inset. B. Recovery time constants and relative amplitudes. Recovery at a given time interval was measured by $I_r$, which was the peak Na$^+$ current relative to the maximal peak Na$^+$ current at a holding potential. A recovery process was fitted with a two-exponential function that is defined by a fast and a slow time constant and two corresponding amplitudes. N=5–19 for each data point. * indicates significant differences between the wild-type and A1180V channels.

Figure 6. Comparison of rate-dependent change in Na$^+$ current between the wild-type and A1180V channels. A. Representative Na$^+$ currents in response to a train of 30 depolarizing pulses,
as illustrated in the inset. Only the 1st and 30th traces are shown. The rate of repetitive pulses was 4 Hz. **B.** Plots of peak Na\(^+\) currents against the number of eliciting voltage pulse. \(I/I_{1st}\): peak Na\(^+\) current relative to peak Na\(^+\) current of the 1st pulse. **C.** Relationship between the rate of repetitive pulses and the change in peak Na\(^+\) current. The change in peak Na\(^+\) current is expressed by \(I_{30th}/I_{1st}\), where \(I_{30th}\) is the peak Na\(^+\) current of the 30th pulse and \(I_{1st}\) is the peak Na\(^+\) current of the 1st pulse. N=5–6 for each data point. * indicates significant differences between the wild-type and A1180V channels.

**Figure 7.** Comparisons of QRS duration and cardiac index between non-carriers and unaffected carriers. **A.** Representative QRS waves in the V\(_3\) lead of a non-carrier (II-13) and an unaffected carrier (III-3) displayed on an expanded time scale. For each individual, an ECG was recorded at rest (heart rate <70 bpm) and immediately after exercise (heart rate >140 bpm); QRS waves recorded under the two conditions were marked with different colors, superimposed, and aligned at QRS onset. **B.** Exercise-induced changes of QRS duration. \(\Delta\)QRS: percent change of QRS duration after exercise. * indicates a significant difference between the non-carrier group (n=8) and the unaffected carrier group (n=6). **C.** Relationship between cardiac index and heart rate of the non-carrier group and the unaffected carrier group. The increase in heart rate was achieved through exercise.
Table. 1 Clinical characteristics, treatment, and results of genetic screening of the pedigree members.

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<th>AF</th>
<th>LVPW (mm)</th>
<th>LAD (mm)</th>
<th>LVEDD (mm)</th>
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<th>Palpitation</th>
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<th>Genotype</th>
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<td>ND</td>
<td>ND</td>
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<td>N/A</td>
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M, male; F, female; D, deceased; N/A, not available; ND, not determined; LVPW, left ventricular posterior wall thickness; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; unfilled blank spaces: no abnormality was found. §: This individual had a PR interval of 202 ms at rest. Individual II-5 is shown as a representative of the non-carrier group of the pedigree. Drugs used in treatment of patients: ACE-I, angiotensin-converting enzyme inhibitor (captopril, 25 mg TID); BB, β-adrenergic blocker (metoprolol, 25–50 mg BID); NVD, nitrate vasodilator (isosorbide dinitrate, 10–20 mg BID); DI, diuretics (hydrochlorothiazide, 25 mg BID–TID; or alternatively, frusemide 40 mg BID was given when heart failure symptoms were severe). ¶: This patient also received intravenous lidocaine when ventricular tachycardia occurred.
Table 2. ECG and cardiac hemodynamic parameters before and after exercise in non-carriers and unaffected mutation carriers.

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ECG parameters: RR, PR, QTc, and QRS stand for corresponding intervals in ms; ΔQRS, percent change of QRS duration after exercise, i.e. \((\text{QRS}_{\text{resting}} - \text{QRS}_{\text{exercise}}) / \text{QRS}_{\text{resting}} \times 100\%\). Cardiac hemodynamic parameters (data in shaded areas) were assessed by echocardiography: HR, heart rate in beats/s; EF, ejection fraction; CI, cardiac index in L/min/m2. *: Significant difference at \(P<0.05\).
when the non-carrier group was compared with the unaffected carrier group. The $P$ values in brackets were obtained by using the Mann-Whitney rank sum test.
Figure 1

A

I aVR V1 V4
II aVL V2 V5
III aVF V3 V6

B

aVL V1

C

LV LA RV LV RA LA
Figure 3
Figure 4
Figure 5

A

B

$\tau_{1,1}$ (ms)  
$\tau_{f,s}$ (ms)  
$A_{1,1}/A_{f,s}$
Figure 6
Figure 7

A) Non-carrier (II-13) and Unaffected carrier (II-3) with Heart rate.

B) ΔQRS (%).

C) CI (L/min/m²) vs Heart Rate (bpm) with Noncarriers and Unaffected carriers.
Molecular and Clinical Characterization of a Novel SCN5A Mutation Associated with Atrioventricular Block and Dilated Cardiomyopathy

Junbo Ge, Aijun Sun, Vesa Paajanen, Shijun Wang, Chunxi Su, Zhiyin Yang, Ying Li, Shaochun Wang, Jianguo Jia, Keqiang Wang, Yunzeng Zou, Lizhi Gao, Kun Wang and Zheng Fan

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