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

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Background—Increased susceptibility to dilated cardiomyopathy 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 of adult onset and dilated cardiomyopathy in a Chinese family.

Methods and Results—Among 32 family members, 5 were initially diagnosed with atrioventricular block after age 30; 4 were studied, 3 of whom later developed dilated cardiomyopathy. 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 \( /H9252 \) 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 although QRS duration decreased with increasing heart rate in noncarrier family members, this change was blunted in unaffected carriers whose ECG and heart function were normal. Resting corrected QT interval of unaffected carriers was significantly longer than that of noncarriers, 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 dilated cardiomyopathy with preceding atrioventricular block. (Circ Arrhythmia Electrophysiol. 2008;1:83-92.)

Key Words: dilated cardiomyopathy • late current • mutation • rate dependence • sodium channel

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 of every 100 000 people.\(^2\) Approximately 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.

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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, which were attributed to electric 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. Case reports describing patients with SCN5A mutations and DCM (in a white family carrying a single-nucleotide polymorphism, S1103Y) or degenerative changes in the conduction system also exist. After refining the genetic mapping of the DCM locus on chromosome 3p22-p25, Olson et al. determined that SCN5A was a candidate gene for DCM; correlation analysis further suggested that 5 SCN5A mutations—T220I, R814W, F851Xs, D1275N, and D1595H—increase susceptibility to myocardial damage and structural disease.

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 3-generation Chinese family. Some family members carrying A1180V suffer from DCM, but not from atrial fibrillation. 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 carried the mutation but had not yet developed the disease and compared them with noncarrier 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 by using the protocols approved by the Ethics Committee of Zhongshan Hospital, Fudan University. The proband (II-1 in Table 1) 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 2D echocardiogram was taken by an echocardiographic machine (Siemens Acuson Sequoia Ecompass III, Siemens, Germany) by using the standard parasternal and apical approaches with the individual in the left lateral decubitus position. We manually measured left ventricular end-diastolic diameter; and unfilled blank spaces, no abnormality was found.
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 2 participating investigators. Each parameter is an average of at least 3 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 polymerase chain reaction from the genomic DNA. Exon 28, because of its large size, was amplified in 3 overlapping fragments. Data Supplement Table I lists all the polymerase chain reaction primers used. The resulting polymerase chain reaction product was sequenced by using an ABI377 automated sequencer (Applied Biosystems, Foster, Calif). Ambiguous bases in, or within 10 bases outside of, an exon were determined by resequencing the exon in the opposite direction or by ABI377 automated sequencer (Applied Biosystems, Foster, Calif). Sequence data of the proband and his affected relatives were also 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 the integrity of the data. 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 us because of palpitation and first-degree AVB. Four years later, the proband developed complete AVB, atrial fibrillation, 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 were engaged in farm work, with the exception of some younger members. The proband’s father (I-1) developed complete AVB and atrial fibrillation at 47 years of age 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, 3 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 DCM 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 (Figure 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 4 affected members and in 6 younger unaffected members of

**Statistical Analysis**

Statistical data are reported as mean±SEM. Statistical differences between wild-type (or noncarrier) and mutation (or carrier) groups were tested by using the Student $t$ 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) 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 the integrity of the data. All authors have read and agree to the manuscript as written.
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 as dilated chambers in the proband (II-1) at age 53, 7 years after pacemaker implantation.

In addition to A1180V, we also detected 4 single-nucleotide variants (Data Supplement Table II) that differed from their counterparts in a reference sequence (GenBank No. 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 nonsynonymous and causes an I244T amino acid change, has not been reported previously. However, it was only found in 4 unaffected individuals who were either parents or siblings, and, therefore, we concluded that it was not related to the disease under study. Haplotype analysis (see Data Supplement Figure) of the variants together with 2 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 (Figure 3A through 3C). A1180V did not significantly affect the channel activation, as can be seen from the voltage dependence and time course of activation (Figures 3D and 4A). However, A1180V induced a negative shift (~4.5 mV) of the steady-state inactivation curve (Figure 4A) but had little influence on the time course of current decay (Figure 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 (Figure 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 (Figure 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 (Figure 6A and 6B). The current loss depended on the rate of the repetitive pulse being greater at a higher pulse rate (Figure 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 electrical properties and hemodynamic function of the heart 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 noncarriers of the family (Figure 2C). Heart rate increase was achieved through exercise. Considering the safety and comfort of the participants, we avoided the use of 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 noncarrier group and the carrier group were not statistically different. However, 3 exceptions exist. First, QRS duration of the unaffected carrier group was shorter. Second, the response of QRS duration to exercise differed between the 2 groups. For the noncarrier 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 noncarrier (II-13). Figure 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 2 groups. Third, the unaffected carrier group demonstrated a statistically longer, though still normal, corrected QT (QTc) interval at rest. After exercise, however, the QTc interval between the 2 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 later, 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 present, in heterozygous form, only in the proband’s pedigree and never in individuals unrelated to the pedigree. Clinically, all carriers in the proband’s generation manifested DCM, whereas unaffected carriers of the pedigree. In the discussion later, we further analyze and interpret these findings to evaluate their implications for the relationship between A1180V and the familial DCM in this pedigree.

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 can subsequently affect cellular pH and Ca²⁺ homeostasis, thus compromising excitation–contraction coupling and energy production mechanisms. However, alternative hypotheses also exist. For example, evidence exists for direct interaction of cytoskeletal proteins with Nav1.5 channels. 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 mech-
Table 2. ECG and Cardiac Hemodynamic Parameters Before and After Exercise in Noncarriers and Unaffected Mutation Carriers

<table>
<thead>
<tr>
<th>ID</th>
<th>A1180V</th>
<th>Sex</th>
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<th>PR</th>
<th>QTc</th>
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<td>393</td>
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<td>132</td>
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<td>78.8</td>
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<td>92.1</td>
<td>81</td>
<td>78</td>
<td>3.24</td>
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<td>113</td>
<td>343</td>
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<td>151</td>
<td>79</td>
<td>5.02</td>
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<td>89.4</td>
<td>73</td>
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<td>152</td>
<td>68</td>
<td>4.17</td>
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ECG parameters: RR, PR, QTc, and QRS stand for corresponding intervals in milliseconds; ∆QRS, percent change of QRS duration after exercise, ie, (QRSresting − QRSexercise)/QRSresting × 100%. Cardiac hemodynamic parameters (data in italics) were assessed by echocardiography: HR, heart rate in beats per second; EF, ejection fraction; CI, cardiac index in L/(min · m²).

*Significant difference at P<0.05 when the noncarrier group was compared with the unaffected carrier group. The P values in brackets were obtained by using the Mann-Whitney rank sum test.
anisms requires determining whether an SCN5A mutation alters the channel current.

When examined in vitro, A1180V channels are distinguishable from wild-type channels in 2 aspects. A1180V channels exhibit (1) a negative shift of steady-state inactivation with a slower recovery that leads to a rate-dependent loss of peak Na\(^+\) current, and (2) an increase in the late Na\(^+\) current. Can these changes in Na\(^+\) current cause myocardial damage? Previously, Grant et al\(^{22}\) reported that \(\Delta K\)1500 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, \(\Delta K\)1500 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 \(\approx 12\%\) of Chinese.\(^{20}\) In our study, heterozygous R1193Q was present in 2 noncarriers, 2 unaffected carriers, and 1 affected carrier (Data Supplement Table II). Thus, the pathogenic significance of the phenotype of this variant is unclear.

Remarkably, we found a better match of the phenotype of A1180V 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 I\(\delta\)C (CaMKII\(\delta\)C) induced a negative shift of steady-state inactivation, slowed recovery from inactivation, and enhanced late Na\(^+\) current. CaMKII\(\delta\)C does not affect channel activation and current density and neither does A1180V. The biophysical profile of cardiac Na\(^+\) channels under the modulation of CaMKII\(\delta\)C qualitatively overlaps with the phenotypic profile of A1180V in all aspects, although quantitatively the effect of CaMKII\(\delta\)C is more prominent. Transgenic mice overexpressing CaMKII\(\delta\)C developed DCM and heart failure,\(^{25}\) and their cardiomyocytes had an abnormally high concentration of cellular Na\(^+\).\(^{24}\) Because A1180V and CaMKII\(\delta\)C share a similar profile in their effect on cardiac Na\(^+\) channels, we postulate that cellular Na\(^+\) concentration and, as a consequence, intracellular Ca\(^2+\) and pH are altered in a heart that expresses A1180V channels. Unfortunately, because CaMKII\(\delta\)C also mediates phosphorylation of Ca\(^2+\) regulatory proteins,\(^{25}\) at present, it is difficult to determine the role of abnormal Na\(^+\) concentration through Na\(^+\) channels in the pathogenesis of DCM on the basis of data obtained from CaMKII\(\delta\)C-transgenic mice.

It needs to be noted that recent studies have shown that the splicing variant Q1077del altered the channel phenotype in some SCN5A mutants and common variants.\(^{26}\) 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 noncarrier group, even though the difference was small and QTc intervals of all individual carriers were still normal. The prolongation disappeared with exercise. Such rate-dependent QT prolongation is typically seen in patients with long-QT syndrome with an SCN5A mutation.\(^{27}\) Thus, we conclude that the prolongation of QTc interval of carriers is substantial, and it is caused by the increased late Na\(^+\) 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.\(^{28}\) In our study, exercise consistently induced QRS shortening in the noncarrier group; interestingly, such shortening was blunted in the unaffected carrier group (Figure 6). We interpret this relative widening of QRS duration to be a consequence of reduced Na\(^+\) 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 noncarrier 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,\(^{29}\) we have considered that the difference may be attributed to fewer
male participants in the unaffected carrier group than the noncarrier group. However, we further found that a similar difference still exists between the female participants of 2 groups, suggesting that short QRS duration may also be a part of the 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\(^+\) current density, possibly because of 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\(^{18}\) reported that disruption of channel ankyrin-G interaction by mutation E1053K blocked Na\(^+\) 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 ECG of the unaffected carriers 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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**CLINICAL PERSPECTIVE**

Dysfunction of cardiac sodium channels due to mutations in the *SCN5A* gene can cause inherited arrhythmogenic conditions such as long-QT syndrome, Brugada syndrome, and familial conduction block. Recent studies have shown that *SCN5A* mutations may also increase susceptibility to dilated cardiomyopathy (DCM), but the underlying mechanism remains unclear. In this study, we investigated 32 members of a family in which 5 members were initially diagnosed with atrioventricular block after age 30; of the 4 we followed over time, 3 later developed DCM. We found a heterozygous single-nucleotide mutation resulting in an amino acid substitution (A1180V) in all followed-up patients and in 6 other younger unaffected members but not in 200 control chromosomes. The mutation caused a negative shift of voltage-dependent inactivation of the cardiac sodium channels with slower recovery leading to a rate-dependent sodium current reduction, and a moderate increase in late sodium current. In parallel, our clinical study revealed QRS widening at high heart rates and QTc prolongation at rest in unaffected carriers. These early signs evident in the ECG of the unaffected carriers precede the development of any detectable conduction system defect and heart function abnormality, and most likely they are congenital. These findings are consistent with the hypothesis that congenital disturbances of cardiac sodium current due to *SCN5A* mutation can substantially contribute to the development of myocardial damage and, ultimately, to DCM. Clinically, the presence of rate-dependent phenotypes in ECGs may provide opportunity for early diagnosis of the DCM-associated mutation even in the absence of a genetic test.
Molecular and Clinical Characterization of a Novel SCN5A Mutation Associated With Atrioventricular Block and Dilated Cardiomyopathy

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