Gain-of-Function Mutation of the SCN5A Gene Causes Exercise-Induced Polymorphic Ventricular Arrhythmias

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Background—Over the past 15 years, a myriad of mutations in genes encoding cardiac ion channels and ion channel interacting proteins have been linked to a long list of inherited atrial and ventricular arrhythmias. The purpose of this study was to identify the genetic and functional determinants underlying exercise-induced polymorphic ventricular arrhythmia present in a large multigenerational family.

Methods and Results—A large 4-generation family presenting with exercise-induced polymorphic ventricular arrhythmia, which was followed for 10 years, was clinically characterized. A novel SCN5A mutation was identified via whole exome sequencing and further functionally evaluated by patch-clamp studies using human embryonic kidney 293 cells. Of 37 living family members, a total of 13 individuals demonstrated ≥50 multiformal premature ventricular complexes or ventricular tachycardia upon exercise stress tests when sinus rate exceeded 99±17 beats per minute. Sudden cardiac arrest occurred in 1 individual during follow-up. Exome sequencing identified a novel missense mutation (p.I141V) in a highly conserved region of the SCN5A gene, encoding the Na_1.5 sodium channel protein that cosegregated with the arrhythmia phenotype. The mutation p.I141V shifted the activation curve toward more negative potentials and increased the window current, whereas action potential simulations suggested that it lowered the excitability threshold of cardiac cells.

Conclusions—Gain-of-function of Na_1.5 may cause familial forms of exercise-induced polymorphic ventricular arrhythmias. (Circ Cardiovasc Genet. 2014;7:771-781.)

Key Words: arrhythmia (heart rhythm disorders) ■ catecholaminergic polymorphic ventricular tachycardia ■ exercise test ■ genetic testing ■ mutation ■ SCN5A

Since 1995, a myriad of mutations in the genes coding for cardiac ion channel subunits or proteins interacting with ion channels were described in patients with inherited forms of arrhythmic disorders, such as congenital long QT syndrome (LQTS), Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT), hence, defining cardiac genetic channelopathies. Most of these disorders have demonstrated a broad genetic and phenotypic heterogeneity. SCN5A is the gene encoding the pore-forming subunit, Na_1.5, mediating the Na⁺ current ($I_{Na}$) of cardiac cells. The arrhythmic phenotypes linked to the mutations in SCN5A are variable, including LQTS type 3, BrS, many forms of conduction defects, atrial fibrillation, sudden infant death syndrome, and dilated cardiomyopathy. More recently, 3 studies have reported on a new SCN5A-dependent clinical presentation called multifocal ectopic Purkinje-related premature contractions caused by the mutation p.R222Q.

Clinical Perspective on p 781
 Premature ventricular complexes (PVCs) emerging at rest in a structurally normal heart are usually considered harmless, whereas PVCs provoked by sympathetic stimulus, such as physical exercise, are regarded as significant finding, necessitating further evaluation. In a proportion of cases, PVCs indicate a risk for lethal ventricular tachycardia or fibrillation. Exercise-induced PVCs, bigeminy, and ventricular tachycardias in structurally normal hearts, characteristic for CPVT, associate with considerable mortality. In fact, lethal arrhythmias may occur in CPVT even before diagnostic exercise-induced symptoms can be verified.

The purpose of this study was to identify the genetic and functional determinants underlying exercise-induced polymorphic ventricular arrhythmia present in a large multigenerational family. Using whole exome sequencing, a novel SCN5A mutation with...
strong cosegregation with the symptomatic patients was found. Functional and computational studies suggest that the mutation-induced biophysical alterations lead to atrial, Purkinje cells, and ventricular hyperexcitability. These findings further expand the spectrum of cardiac arrhythmias caused by SCN5A variants.

Methods
A Finnish family with 37 living members in 4 generations was evaluated (Figure 1). Altogether 31 family members underwent cardiological examination, including electrocardiographic tests and cardiac ultrasonography between years 2002 and 2003. In 2013, at the end of the follow-up period, all family members were recontacted and invited for assessment of their symptoms and possible cardiovascular interventions. Informed consent was obtained from all patients. The study was performed in accordance with the Helsinki Declaration and was approved by the local ethical review board.

Electrocardiographic and Ultrasonographic Examinations
Standard ECG was recorded at rest (50 mm/s, 0.1 mV/mm). QT interval was measured from lead II and adjusted for heart rate (QTc) according to the Fridericia’s correction formula. Bicycle ergometer test was performed with continuous 12-lead ECG recording. The initial load was 30 W, followed by increments of 15 W each minute. The total number of PVCs and the maximum number of consecutive PVCs in ventricular salvos were counted during work phase.

A 24-hour ambulatory ECG was recorded on an outpatient basis. Rhythm, mean heart rate, number of ventricular premature complexes, and number and maximum length of ventricular tachycardias (≥3 consecutive ventricular complexes) were calculated. Echocardiography was performed using parasternal long- and short-axis and apical 4-chamber views. Aortic root dimension was measured at the sinus valsalva level. Left ventricular dimensions and wall thickness were measured from the M-mode recordings. Doppler echocardiography was used to exclude any valvular stenosis or regurgitation.

Phenotypic Classification of Family Members
Patients with ≥50 polymorphic PVCs or >10 polymorphic PVCs per minute or ventricular tachycardia during exercise stress test (ET) were considered affected. Individuals who were unable to perform an ET were classified as unknown.

![Figure 1. The Na,1.5 p.I141V mutation. A. The pedigree, phenotypes, and mutation status of family members. The presence of ≥50 premature ventricular complexes (PVC) during an exercise stress test (ET) is indicated by a symbol with a half-filled left-side, the presence of ectopic atrial rhythm is indicated by a filled upper right quadrant and atrial fibrillation by a filled lower right quadrant. A white symbol indicated normal ECG, whereas individuals without ET are indicated with a grey symbol. The presence of the I141V mutation is specified by a + or –. B. Targeted sequencing confirmed a heterozygous missense mutation, chr3:38,663,952 T->C, cosegregating with multiple PVCs during ET. Representative traces from 4 of the sequenced family members are shown. C. Multiple protein sequence alignments showing the conservation of isoleucine at codon 141 of the Na,1.5 protein in different vertebrate species. D. A schematic picture of the Na,1.5 protein structure, indicating the location of the I141V-mutation in domain I.](http://circgenetics.ahajournals.org/)

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Sequencing of Candidate Genes

Mutations in RYR2 were excluded by sequencing its all translated exons and exon-intron boundaries in the DNA sample of the proband (AMC, University of Amsterdam, Netherlands).

Whole exome sequencing of 8 affected pedigree members was performed using the NimbleGen SeqCapEZ sequence capture technology (www.nimblegen.com/products/seqcap/ez/index.html). The exome targets of the patients’ DNA were captured with the NimbleGen SeqCap Ez Human Exome Library v2.0, followed by sequencing with the Illumina Genome Analyzer-Ix platform at the Technology Center at the Institute for Molecular Medicine Finland. The alignment to human reference genome hg19 and variant calling of chromosomal regions with a sequence-coverage of 25x was performed using the variant calling pipeline of the Institute for Molecular Medicine Finland. The data were filtered to capture only rare coding variants, that is, variants giving rise to missense, nonsense, splice site, or frame shift mutations either with minor allele frequencies <0.005 or not at all present in the 1000 genomes catalogue of human genetic variation (http://browser.1000genomes.org/index.html). The presence of a chr3:38,663,952 T>C variant in the SCN5A gene, initially identified by exome sequencing, was assessed in all affected and unaffected family members by direct Sanger sequencing using forward primer 5’TATCTCCTGCCGATATTCTGG 3’ and reverse primer 5’CTTGGAGACCTGTTTTAGG 3’. The sequencing reactions were run on an Applied Biosystems 96-capillary 3730xl DNA Analyzer and analyzed with the Variant Reporter software (Applied Biosystems, US) at the Technology Center at the Institute for Molecular Medicine Finland. The predicted effect of the rare coding SCN5A variant identified was analyzed by PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/).

Microsatellite Genotyping

Genome-wide linkage mapping was performed using ABI PRISM Linkage mapping Set MD10 (Applied Biosystems). Standard PCR protocols were used for amplification of microsatellite fragments using 10 ng of genomic DNA as a template. The fluorescently labeled PCR products were separated using ABI3730 (Applied Biosystems) automated electrophoresis system and the genotype calls were made using GeneMapper3.7 software. Two independent reviewers verified all allele calls, and any discrepancies were resolved. Genotypes were checked for violation of Mendelian segregation of alleles using PEDCHECK.11

Linkage Analysis

Linkage analysis using exercise-induced frequent polymorphic ventricular premature complexes during an exercise test as a dichotomized trait was performed using the program Merlin (http://www.sph.umich.edu/csg/abecasis/merlin/index.html). The analyses were run applying an affected-only approach in which the phenotype of all unaffected family members was assumed be unknown, and the residual capacitive currents were cancelled using a B94 protocol. The cells were bathed with an extracellular solution containing (in mmol/L) NaCl 50, NMDG-Cl 80, CsCl 5, MgCl2 1.2, CaCl2 2, HEPES 10, and glucose 5. The pH was adjusted to 7.4 with CsOH. Glass pipettes (tip resistance, 1.5–3 mol/LΩ) were filled with an intracellular medium containing (in mmol/L) CsCl 60, aspartic acid 50, CaCl2 1, MgCl2 1, HEPES 10, EGTA 11, and NaATP 5. pH was adjusted to 7.2 with CsOH. For the window current measurements, the presented recordings are normalized to the peak current obtained at −20 mV and are tetrodotoxin (TTX)-subtracted (30 μM).

All products were purchased from Sigma, except TTX, which was obtained from Alomone.

Computer Simulations of $I_{Na}$ Action Potentials (AP), and Conduction

The $I_{Na}$ and action potentials were simulated using the 10 Tusscher–Noble–Panfilov (TNNP) human ventricular cell model,12 the Courtemanche–Ramirez–Nattel human atrial cell model,13 and the Stewart–Aslanidi–Noble–Boyett–Zhang Purkinje cell model.14 In all 3 models, $I_{Na}$ is represented according to a Hodgkin–Huxley formalism, $I_{Na}=g_{Na}(m^3h(V–E_{Na}))$, where $g_{Na}$ is the maximal conductance of $I_{Na}$, $m^3$ represents 3 activation gates, h and f are inactivation gates, $V$ is the membrane potential, and $E_{Na}$ is the Nernst potential of sodium. The $I_{Na}$ formulation in the TNNP and Stewart–Aslanidi–Noble–Boyett–Zhang models, which are based on human $I_{Na}$ recordings, are equivalent up to the maximal conductance of $I_{Na}$ but they differ in terms of steady state values and time constants of the different gates from the $I_{Na}$ formulation in the Courtemanche–Ramirez–Nattel model, which is based on that of Luo and Rudy.15 Table 1 presents the modifications of $I_{Na}$ that were introduced in the 3 models to match the experimental data.

Action potential propagation was simulated in a 1 cm long fiber based on the equation

$$\frac{\partial I_{Na}}{\partial t} = -I_{Na} + D \frac{\partial^2 I_{Na}}{\partial x^2},$$

where $I_{Na}$ is the sum of the ionic currents normalized to cell capacitance and $D$ is the diffusion constant, which depends on intra- and intercellular conductance and surface-to-volume ratio. For all 3 models, $D$ was set to 0.00154 cm²/ms as in the original TNNP model12,16 which corresponds to the normal level of gap junctional coupling in the direction of myocardial fibers. Using the same $D$ allows a direct comparison of conduction between the 3 models. Gating variables were integrated using the method of Rush and Larsen and the other model variables using the forward Euler algorithm, with $\Delta t=0.01$ cm and $\Delta t=0.005$ ms. The fiber was stimulated at 1 extremity and conduction velocity was computed using linear regression of activation times (occurrence of dV/dt(dmax) over a segment extending from 25% to 75% of fiber length. Of note, the computer simulations have the limitation that the models did not incorporate the effects of adrenergic stimulation. Of note, in the current study, we did not incorporate any variability in the computer simulations. All the used values are the arithmetic means of the given parameters.
sequent ETs revealed the same in 3 additional individuals, all with PVCs during exercise in 10 and the sub-
greater than or equal to 50 PVCs during exercise. In all of them, the QRS complex morphology and duration, ST segment,
interval, QRS complex morphology and duration, ST segment, and T-wave pattern were normal in all during sinus rhythm.

For both models

\[ \frac{d m}{dt} = \left( V_{m} - m \right) / \tau_m \]

\[ \frac{d h}{dt} = \left( h - h_{\infty} \right) / \tau_h \]

WT denote the formulation of the original models. The shift parameters \( \alpha \) and \( \sigma \) were determined based on experimental data.

CRN, Courtmanche-Ramirez-Nattel; SANNBZ, Stewart–Aslanidi–Noble–Boytz-Zhang; TNPP, Tusscher–Noble–Bazin–VanPatten; and WT, wild type.

### Results

The structure of the Finnish family segregating the exercise-induced arrhythmia syndrome is shown in Figure 1A, and the findings of the electrocardiographic and cardiac ultrasonography studies are presented in Table 2. Of those alive in the 4-generation family, a total of 6 individuals had experienced a syncopal spell. One case of sudden cardiac death occurred during prospective follow-up. Family member II-1 had died at the age of 2 years. None of the family members had a documented coronary artery disease or a history of angina pectoris.

### Data Analysis and Statistical Methods

Currents were analyzed with Clampfit software (Axon Instruments, Inc). Data were analyzed using a combination of pClamp10, Excel (Microsoft), and Prism (graphpad).

Comparisons between groups were performed with 2-tailed Student's t test or ANOVA for normally distributed parameters. The Kolmogorov–Smirnov test was used to test the normality of the analyzed experimental data. Data are expressed as mean±SD. A P value <0.05 was considered significant.

### ECG Registrations

Of 16 adult Na\(_{\text{1.5}}\) p.I141V mutation carriers, 5 presented with sinus rhythm, 1 with atrial flutter, and 11 with ectopic atrial rhythm in their resting ECG (Table 2; Figure 2A and 2B). Nodal escape rhythm was seen occasionally (Figure 2C). PVCs were not present in resting ECG in any of the family members at first examination. The sinus rate at rest was higher in Na\(_{\text{1.5}}\) p.I141V mutation carriers than in noncarriers (Table 3). PQ and QT\(_s\) intervals, QRS complex morphology and duration, ST segment, and T-wave pattern were normal in all during sinus rhythm.

A total of 32 subjects were studied by repeated ETs. The first ET showed \( \geq 50 \) PVCs during exercise in 10 and the subsequent ETs revealed the same in 3 additional individuals, all carriers of Na\(_{\text{1.5}}\) p.I141V mutation. During the ETs, the ventricular arrhythmias progressively became more complex and abundant in parallel with the increasing sinus rate; examples

### Cardiac Ultrasonography

In cardiac ultrasonography, all Na\(_{\text{1.5}}\) p.I141V mutation carriers showed normal left ventricular end-diastolic diameter and index (48±3 mm and 24.5±2.8 mm/m², respectively). Aortic root diameter was \( \geq 44 \) mm in 3 of them.

### Detection of the SCN5A Mutation

By using whole exome sequencing, we detected a novel missense mutation, (chr3:38,663,952 T->C) in SCN5A, encoding for the Na\(_{\text{1.5}}\) sodium channel protein, in addition to 2 rare coding mutations, that is, rs148604148 (missense, MAF 0.002) in XLYB, encoding for xylulokinase homolog (H. influenzae) and rs141040660 (synonymous, MAF 0.0005) in ANO10, encoding for anotamin 10, that were shared by all 8 sequenced individuals. The 3 genes are located at 5 Mb distance from each other on chromosome 3p22.2, but only SCN5A is expressed in cardiac tissue and is known to be associated with several arrhythmia disorders, including congenital LQTS type 3, BrS, and idiopathic ventricular fibrillation.

Because the exome sequencing resulted in low coverage across chr3:38,663,952, ie, 3x to 9x, the initial analyses showed the presence of the chr3:38,663,952 T->C in SCN5A, encoding for the Na\(_{\text{1.5}}\) sodium channel protein, in addition to 2 rare coding mutations, that is, rs148604148 (missense, MAF 0.002) in XLYB, encoding for xylulokinase homolog (H. influenzae) and rs141040660 (synonymous, MAF 0.0005) in ANO10, encoding for anotamin 10, that were shared by all 8 sequenced affected family members. Nonetheless, targeted sequencing of the complete family showed that all 13 affected pedigree members in addition to 4 unaffected family members and 3 children with unknown phenotype carried the chr3:38,663,952 T->C mutation (representative sequence traces from 4 family members are presented in Figure 1B). Based on PolyPhen2 and SIFTanalyses, the mutation, which results in an isoleucine to valine substitution at position 141 of the Na\(_{\text{1.5}}\) protein (p.I141V), was classified as possibly damaging with a score of 0.46 and deleterious with a score of 0.00, respectively. Moreover, the mutation seems to be unique to this family. It is not present in the published 1000 genomes data set, of which 83 are Finnish subjects, neither was it present in exome sequence data from 580 unrelated Finnish subjects (unpublished observation), further indicating that this sequence variant is rare. Finally, we performed linkage analyses to assess the probability that all affected individuals share a mutation. The chr3:38,663,952 T->C variant yielded a 2-point LOD-score of 3.56 (conservatively classifying only individuals with >50 exercise-induced polymorphic complexes as affected), whereas genome-wide linkage analyses of the whole pedigree utilizing microsatellite genotypes indicated that the chr3:38,663,952 T->C variant resided in the only chromosomal region displaying complete cosegregation with the arrhythmia phenotype. No mutations were found in the genes RYR2 and CASQ2.
Effects of p.I141V Mutation on Na\textsubscript{1.5} Channel Function in Human Embryonic Kidney 293 Cells

To investigate the functional consequences of the p.I141V mutation on the Na\textsuperscript{+} channel activity, we used the whole-cell configuration of the patch-clamp technique. The presence of the mutation did not modify the Na\textsuperscript{+} current density (Table A in the Data Supplement). The voltage-dependence of steady state activation was shifted toward more negative potentials (−7 mV) in the presence of the mutation (P<0.01; Figure 3A and 3B; Table A in the Data Supplement). For a given command voltage, the activation and inactivation kinetics were accelerated in the mutant channel (Figure 3C and 3D). However, no significant differences were observed regarding the voltage dependence of steady state inactivation (Figure 3B; Table A in the Data Supplement), the recovery from fast inactivation (Figure 3E), and the onset and recovery from slow inactivation (Figure QA and IB in the Data Supplement).

Based on the negative shift of the steady state activation curve, we predicted an increase of the window sodium current (Figure 3B, inset). To investigate this window current, we examined the responses of cells transfected with Na\textsubscript{1.5} WT and p.I141V channels to a ramp voltage protocol, in which membrane potential was gradually changed between −100 and +50 mV at 0.5 mV/ms. Compared with the WT window current, the p.I141V window current exhibited a larger peak, and this peak was shifted toward more negative potentials (Figure 3F; Figure IC and ID in the Data Supplement).

The Importance of the p.I141 Residue on Na\textsubscript{1.5} Channel Function

The isoleucine 141 (I141) amino acid is highly conserved across species- and voltage-gated sodium channels isoforms (Figure 1C and 1D). The importance of the p.I141 position on Na\textsubscript{1.5} function was investigated by mutating the nearest and highly conserved isoleucine 137 to valine. Interestingly, this particular substitution (p.I137V) did not modify any of the Na\textsubscript{1.5} channel properties investigated (Figure II in the Data Supplement).

### Table 2. Clinical Data of Family Members Carrying the Na\textsubscript{1.5} p.I141V Mutation

<table>
<thead>
<tr>
<th>Family Member No.</th>
<th>Age</th>
<th>Sex</th>
<th>Exercise-Induced PVCs</th>
<th>Syncopal Spells</th>
<th>Ectopic Atrial Rhythm</th>
<th>Atrial Fibrillation</th>
<th>Threshold Heart Rate per Minute for PVCs</th>
<th>Number of PVCs During ET</th>
<th>Longest Salvo of PVCs (beats)</th>
<th>Total No. of PVCs/24 h</th>
<th>Treatment at the Last Follow-Up Visit 2013</th>
</tr>
</thead>
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<tr>
<td>II-2</td>
<td>80</td>
<td>F</td>
<td>Positive</td>
<td>No</td>
<td>+</td>
<td>Paroxysmal</td>
<td>93</td>
<td>231</td>
<td>3</td>
<td>1715</td>
<td>Metoprolol 47.5 mg</td>
</tr>
<tr>
<td>II-4</td>
<td>77</td>
<td>M</td>
<td>Positive</td>
<td>No</td>
<td>−</td>
<td>Chronic</td>
<td>110</td>
<td>100</td>
<td>2</td>
<td>11439</td>
<td>Bisoprolol 5 mg</td>
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<tr>
<td>II-7</td>
<td>74</td>
<td>M</td>
<td>Positive</td>
<td>No</td>
<td>+</td>
<td>…</td>
<td>90</td>
<td>83</td>
<td>4</td>
<td>241</td>
<td>Bisoprolol 2.5 mg</td>
</tr>
<tr>
<td>II-10</td>
<td>70</td>
<td>F</td>
<td>Positive</td>
<td>No</td>
<td>+</td>
<td>…</td>
<td>77</td>
<td>219</td>
<td>8</td>
<td>12470</td>
<td>Propranolol 40 mg, Bisoprolol 5 mg BID</td>
</tr>
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<td>II-12</td>
<td>65</td>
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<td>No</td>
<td>+</td>
<td>Chronic</td>
<td>74</td>
<td>483</td>
<td>3</td>
<td>1399</td>
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<td>No</td>
<td>−</td>
<td>Paroxysmal</td>
<td>126</td>
<td>630</td>
<td>8</td>
<td>5601</td>
<td>Metoprolol 142.5+47.5 mg</td>
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<td>III-1</td>
<td>61</td>
<td>M</td>
<td>Negative</td>
<td>No</td>
<td>+</td>
<td>…</td>
<td>N/A</td>
<td>6</td>
<td>1</td>
<td>187</td>
<td>None</td>
</tr>
<tr>
<td>III-3</td>
<td>4</td>
<td>M</td>
<td>Positive</td>
<td>No</td>
<td>−</td>
<td>Atrial flutter</td>
<td>95</td>
<td>492</td>
<td>2</td>
<td>N/A</td>
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<tr>
<td>III-5</td>
<td>52</td>
<td>F</td>
<td>Positive</td>
<td>Yes</td>
<td>+</td>
<td>…</td>
<td>84</td>
<td>508</td>
<td>5</td>
<td>1646</td>
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<td>50</td>
<td>M</td>
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<td>Yes</td>
<td>+</td>
<td>Chronic</td>
<td>108</td>
<td>637</td>
<td>5</td>
<td>336</td>
<td>Bisoprolol 7.5 mg BID</td>
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<td>48</td>
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<td>Yes</td>
<td>+</td>
<td>…</td>
<td>120</td>
<td>346</td>
<td>6</td>
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<td>Bisoprolol 2.5 mg b.i.d</td>
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<td>37</td>
<td>M</td>
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<td>No</td>
<td>+</td>
<td>…</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td>III-19</td>
<td>34</td>
<td>F</td>
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<td>No</td>
<td>−</td>
<td>…</td>
<td>104</td>
<td>540</td>
<td>3</td>
<td>9925</td>
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<td>M</td>
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<td>No</td>
<td>−</td>
<td>…</td>
<td>105</td>
<td>352</td>
<td>2</td>
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<td>−</td>
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<td>N/A</td>
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<td>1</td>
<td>N/A</td>
<td>None</td>
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<td>M</td>
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<td>No</td>
<td>+</td>
<td>…</td>
<td>108</td>
<td>441</td>
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<td>11</td>
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<tr>
<td>IV-13</td>
<td>4</td>
<td>M</td>
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<td>…</td>
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<td>M</td>
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<td>+</td>
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<td>N/A</td>
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<td>9</td>
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<td>−</td>
<td>…</td>
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<td>N/A</td>
<td>4</td>
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<tr>
<td>V-1</td>
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<td>M</td>
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<td>No</td>
<td>N/A</td>
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</table>

ET indicates exercise stress test; N/A, not available; and PVC, premature ventricular complex.

*Deceased.
To investigate the functional consequences of the cardiac hyperexcitability because of the p.I141V mutation of Na\_1.5, we incorporated the p.I141V Na\_1.5 properties observed experimentally into the atrial Courtemanche–Ramirez–Nattel, the ventricular TNNP, and the Purkinje Stewart–Aslanidi–Noble–Noble–Boyett–Zhang cell models. The $I_{Na}$ formulations in all models were modified to reproduce the biophysical changes caused by the p.I141V mutation (see Methods and Figure 4A–4C). In the $I_{Na}$ formulations of all models, the effects of the p.I141V mutation were simulated by shifting the voltage dependence of the steady state equilibrium $m_\infty$ and the time constant $\tau_m$ of the $m$ gates by an equal amount to recapitulate the shift of the steady state activation curve and the acceleration of activation and by shifting the voltage dependence of the closing rate constant $\beta_h$ of the $h$ gate to recapitulate the acceleration of fast inactivation with no change in the recovery from inactivation. The $j$ gate was left unchanged. The formulations used are summarized in Table 1.

Simulations were run using the 3 cell models for WT, using both heterozygous and homozygous p.I141V genotypes, and the strength–duration curves were constructed. In the 3 models, a lower excitation threshold for action potential generation (pacing rates, 1 and 2.5 Hz for the atrial and ventricular models and 2.5 Hz for the Purkinje model) was observed in the homozygous and heterozygous p.I141V genotypes compared with the WT (Figure 4D–4I). Interestingly, at both pacing rates, the strength–duration curve for

### Table 3. Electrocardiographic Parameters at Rest During the Initial Evaluation in 2002 to 2003

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SCN5A-positive, n=12</th>
<th>SCN5A-negative, n=17</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (range)</td>
<td>42±18 (21–69)</td>
<td>43±15 (17–69)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, (male/female)</td>
<td>7/5</td>
<td>9/8</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, min(^{-1}) (range)</td>
<td>79±9 (63–91)</td>
<td>66±6 (54–75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P, ms</td>
<td>100±11</td>
<td>100±12</td>
<td>NS</td>
</tr>
<tr>
<td>PQ, ms</td>
<td>154±29</td>
<td>159±22</td>
<td>NS</td>
</tr>
<tr>
<td>QRS, ms</td>
<td>89±15</td>
<td>91±9</td>
<td>NS</td>
</tr>
<tr>
<td>QT, ms</td>
<td>378±35</td>
<td>390±24</td>
<td>NS</td>
</tr>
<tr>
<td>QTc, ms (range)</td>
<td>413±24 (383–462)</td>
<td>401±19 (360–430)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are from subjects without medication. NS indicates not significant.
Swan et al  SCN5A Causing Exercise-Induced Polymorphic VT 777

the heterozygous genotype was close to the curve for the homozygous genotype. In the Purkinje cell model, we observed that the p.I141V mutation accelerates the rate of spontaneous activity, even in the heterozygous state (Inset Figure 4F).

Conduction velocity was investigated in fibers of Courtemanche–Ramirez–Nattel, TNNP, and SANNBZ cell models (pacing rates, as described above). As shown in Figure 4J–4L, the presence of the p.I141V mutation in homozygous and heterozygous states accelerated atrial and ventricular conduction in a similar manner at 1 and 2.5 Hz, and it accelerated conduction in the Purkinje fiber at 2.5 Hz.

Clinical Follow-Up
During a follow-up of 10 years, altogether 38% of 16 adult Na1.5 p.I141V carriers have either permanent (n=4) or paroxysmal (n=2) atrial fibrillation. At the end of the follow-up period, β-antiadrenergic medication was used by 10 individuals, 1 of whom was also using amiodarone. An implantable defibrillator (ICD) was implanted in another tertiary care center to 1 family member (II:14) because of nonsustained ventricular tachycardias. Left ventricular dilatation or systolic heart failure was detected in none of the family members during follow-up. The dilated aortic root had not required surgical intervention in any of the cases during follow-up.

Another family member (III-3) died during the follow-up 3 years after a mitral valve replacement. He had been followed from the age of 36 because of frequent multiformic PVCs, paroxysmal atrial fibrillation, and mitral valve prolapse. Perioperative pulmonary vein isolation was unsuccessful in eliminating AF. A

Figure 3. Experimental effects of p.I141V mutation on Nav1.5 channel in human embryonic kidney 293 (HEK293) cells. A, Current traces obtained with a current/voltage protocol (see inset) from Na1.5-WT and p.I141V transfected cells. Bold traces are the Na+ current at –40 mV. B, Steady-state activation (WT, n=8; p.I141V, n=9) and inactivation curves (WT, n=8; p.I141V, n=9). Activation properties were determined from I/V relationships by normalizing peak \( I_{\text{Na}} \) to driving force and maximal \( I_{\text{Na}} \). Parameters for the voltage-dependence steady state of activation and steady state of inactivation (20-ms test pulse to –10 mV after a 500 ms conditioning prepulse) are summarized in Table in the Data Supplement. C, Sodium current time-to-peak values were used to evaluate the activation kinetics (WT, n=8; p.I141V, n=9). D, Fast inactivation time constants were measured by fitting the inactivation phase of the Na+ current to a single exponential function for WT and p.I141V (WT, n=8; p.I141V, n=9). For C and D, a 2-way ANOVA test followed by a Bonferroni correction was used to compare point-by-point the measured activation and inactivation: ***P<0.001 vs WT. E, Recovery from fast inactivation was measured using the inset twin-pulse protocol (WT, n=8; p.I141V, n=9). F, Averaged normalized WT (n=13) and p.I141V (n=14) tetrodotoxin-sensitive window currents obtained with a 300 ms depolarizing-voltage ramp from –100 and +50 mV (0.5 mV/ms), normalized to the peak current at –20 mV recorded in the same cell. Represented in panel F are the averaged normalized window current traces at the potential range between –80 and +10 mV.
A ventricular pacemaker was implanted and atrioventricular node ablation was performed resulting in temporary atrioventricular block. During follow-up, the patient experienced a syncopal spell 3 times. A month later, sudden cardiac arrest occurred at home during stressful conditions; patient was resuscitated from ventricular fibrillation but died few days later.

Additionally, we repeated ETs to all family members (n=21, including both mutation carriers and family members not carrying the Nav1.5 p.I141V mutation) whose ET was normal at the first examination. In the follow-up test, 3 individuals (V-7, III-11, and II-7) showed ≥50 PVCs at the age of 31, 45, and 69 years, respectively. They were all carrying the Na1.5 p.I141V mutation.

The ambulatory 24-hour ECG recording was performed at the end of follow-up period in 14 Na1.5 p.I141V mutation carriers to assess the burden of AF. This revealed 1 additional patient (II:14) with AF, which would not have been diagnosed during ETs. At resting heart rates, PVCs were absent or their frequency was low in most Nav1.5 p.I141V mutation carriers but their incidence increased concomitantly with physical activity and increasing sinus rate as illustrated in Figure III in the Data Supplement.
Discussion
In the present study, we describe a novel missense mutation in a highly conserved region of the SCN5A gene, which associates with an autosomal dominantly inherited arrhythmic phenotype in a large multigenerational Finnish family. This phenotype is characterized by exercise-induced polymorphic PVCs, bigeminy, and nonsustained polymorphic ventricular tachycardia in structurally normal hearts. Arrhythmias disappear after cessation of exercise.

The affected family members displayed widespread cardiac hyperexcitability features manifesting as increased sinus rate, atrial ectopic rhythm, and atrial and nodal tachyarrhythmias. In the current SCN5A-linked family, the propensity to atrial arrhythmias appeared to increase with age, resulting in paroxysmal and eventually chronic AF in many cases. Also, the number of ventricular complexes during exercise increased in some patients during follow-up, further supporting a progressive nature of the disorder. The earliest age of onset of ventricular arrhythmias among mutation carriers cannot be exactly determined using data from this single family. The youngest mutation carrier capable of performing an ET was 11 years. Underscoring the diagnostic challenge, this boy did not exert multiple polymorphic PVCs during the clinical ET, in spite of that, his 24-hour ambulatory ECG recording revealed 1210 PVCs associated with physical exercise. Even if it is clear that the p.I141V mutation is not fully penetrant, the penetrance of the PVC phenotype is high, reaching 81% when considering only adult mutation carriers.

Evidence for Na_1.5 p.I141V Causality in Exercise-Induced Polymorphic Arrhythmias
Although sequencing 2 putative genes (RYR2 and CASQ2) in affected members of the current family failed to identify any coding mutations, whole exome sequencing revealed a previously unknown missense mutation in SCN5A, which cosegregated with the arrhythmic phenotype. The accumulated evidence strongly supports the assumption that this variant is indeed functional and causes arrhythmia in the family investigated. First, the SCN5A gene resides in the only chromosomal region showing complete cosegregation with the ventricular arrhythmia phenotype. Second, the identified mutation results in an amino acid change p.I141V of the Na_1.5 protein, which in silico is predicted to be possibly damaging. Third, the mutation resides in a region of the SCN5A gene that is highly conserved between species and Na_1 homologues. Fourth, patch-clamp experiments show that the introduction of the mutation causes hyperexcitability of the Na_1.5 channels. Finally, computational analyses of I_\text{Na} and AP in single cells imply that the biochemical changes caused by the p.I141V mutation both include an acceleration of the spontaneous depolarization rate in the Purkinje cell model, which is similar to what was reported for a multifocal ectopic Purkinje-related premature contraction–linked p.R222Q mutation by Mann et al, and a reduction of the threshold for cardiac cell excitability.

Isolecine in the position 141 of the Na_1.5 protein is highly conserved, not only across species, but also between Na_1 isoforms. Interestingly, Na_1.4 p.I141V and Na_1.7 p.I136V mutations, that are homologous to the Na_1.5 p.I141V mutation, have also been associated with hyperexcitability-dependent inherited disorders, that is, with myotonia and erythromelalgia. Both these previously described mutations induce similar modifications of the biophysical properties of the sodium voltage gated channels as was observed for the Na_1.5 p.I141V mutation in the current study. Taken together, these findings strongly support the pathogenicity of this novel SCN5A missense variant.

Gain-of-Function Mutations in Na_1.5 and the Clinical Phenotypes
Gain-of-function mutations in Na_1.5 have previously been associated with several hereditary forms of arrhythmia, but also with the more recently described multifocal ectopic Purkinje-related premature contraction with cardiomyopathy, that is linked to the presence of the p.R222Q mutation in Na_1.5. In the current study, the clinical phenotype, associated with the p.I141V mutation, was characterized by a distinct hyperexcitability without evidence of conduction abnormalities or QT interval prolongation. Although the p.I141V and the p.R222Q mutation both associate with atrial arrhythmias, the clinical characteristics of the individuals carrying the p.I141V mutation differ from the phenotype described in p.R222Q-carriers. Although exercise provoked ventricular arrhythmias in p.I141V-carriers, p.R222Q-carriers typically manifest arrhythmias at resting heart rates. Moreover, carriers of p.R222Q show frequent PVCs, with both left and right bundle-branch block patterns triggered from Purkinje fibers at low heart rates, but disappearance of PVCs during exercise, and dilated cardiomyopathy. In p.I141V mutation carriers, the burden of PVCs was much lower, which may explain why they did not display any abnormalities in ventricular structure or contractility.

In spite of the above described dissimilarities between the clinical phenotypes, the differences in the cellular phenotypes associating with the p.I141V and p.R222Q mutations were more subtle. Although the p.I141V mutation did not modify the voltage dependence of inactivation, it did shift the activation curve to negative potentials and led to an increase and shift of the sodium window current. In contrast, the p.R222Q mutation affects the voltage dependence of both activation and inactivation, resulting in a negative shift of the sodium window current. To bear in mind, in vitro phenotypes observed in heterologous expression systems may not always reflect the clinical phenotypes induced by a mutation. As an example, although many studies have reported several varied phenotypes associating with a SCN5A p.D1275N missense mutation, for example, atrial standstill, dilated cardiomyopathy with conduction disease, atrial flutter/fibrillation, sick sinus syndrome, and ventricular dilation and dysfunction, the mutation does not induce marked Na_1.5 dysfunction in vitro. However, when studied in genetically engineered mice, in vivo, the mutation generates extensive aberration of channel function, underscoring that functional phenotypes may be influenced substantially by the experimental systems used.

Overall, the complex pathways underlying the genotype–phenotype relationship in hereditary arrhythmia syndromes are poorly known, and the reasons for incomplete penetrance and variable expressivity remain obscure. Pleiotropy has been associated with SCN5A mutations in particular. Although distinct clinical phenotypes have been ascribed to different mutations in the SCN5A gene, for example, LQTS, BrS, and cardiomyopathy by others and the present exercise-induced phenotype by us,
some mutations also display a substantial degree of variability within families. For example, the affected family members of a large family segregating a SCN5A-1795insD presented with a wide array of clinical manifestations, including sinus node dysfunction, bradyarrhythmia, conduction disease, BrS, and LQTS.23,24

Mechanistic Considerations

The mutation-induced alterations of Na,1.5 properties and simulation studies support the concept that the increase in window current lead to a ventricular and atrial hyperexcitability. It is interesting to note that administration of flecainide 2 mg/kg IV to patients II-10 and III-19 resulted in a significant reduction of PVCs during an exercise stress (from 219 and 540 to 17 and 0, respectively). Also, in a previous study, flecainide has been shown to induce a dramatic reduction in PVCs and recovery of normal function, bradycardia, conduction disease, BrS, and LQTS.23,24

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Premature ventricular complexes constitute a diagnostic challenge because in a small proportion of patients, they may indicate the risk of lethal ventricular tachycardia or fibrillation. Exercise-induced premature ventricular complexes, bigeminy, and ventricular tachycardias in structurally normal hearts are characteristic of the catecholaminergic polymorphic ventricular tachycardia and are associated with considerable mortality. A correct presymptomatic diagnosis by genetic testing is particularly valuable because lethal arrhythmias may occur in catecholaminergic polymorphic ventricular tachycardia even before diagnostic exercise-induced symptoms can be verified, at least in carriers with mutations of the ryanodine receptor type 2 gene. We demonstrate in this investigation that a mutation in the cardiac sodium channel gene SCN5A can also cause exercise-induced polymorphic ventricular tachycardias. Similar to catecholaminergic polymorphic ventricular tachycardia, physical exercise provokes premature ventricular complexes and nonsustained polymorphic ventricular tachycardia that disappear after cessation of exercise. Unlike in catecholaminergic polymorphic ventricular tachycardia, also the atria are prone to ectopic activity and tachyarrhythmias. Our findings could help the clinician to differentiate these exercise-provoked ventricular arrhythmias from those associated with ryanodine receptor type 2 and calsequestrin 2 mutations. Awareness of the potential contribution of SCN5A to inherited exercise-induced polymorphic ventricular tachyarrhythmias could facilitate the identification of more mutation carriers and assist in determining their prognostic significance. Patients with the exercise-induced polymorphic ventricular arrhythmias could be tested also for the SCN5A gene, particularly if ryanodine receptor type 2 and calsequestrin 2 have tested negative. In general, the present study demonstrates the importance of genotypic and phenotypic classification of cardiac arrhythmias which have similar clinical presentations but actually may have different prognosis and require different therapeutic approaches.
### Supplemental Table

#### Table A: Electrophysiological characteristics of Nav1.5-I141V

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>p.I141V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak current at -20 mV (pA/pF)</td>
<td>-166.9±19.7; n = 8</td>
<td>-195.5±9.1; n = 9</td>
</tr>
<tr>
<td>Activation</td>
<td>( V_{1/2} = -28\pm1.2; ) K = 6.7±0.4; n = 8</td>
<td>( V_{1/2} = -35\pm1.3^{***}; ) K = 6.4±0.3; n = 9</td>
</tr>
<tr>
<td>Inactivation</td>
<td>( V_{1/2} = -75.8\pm2; ) K = 6.3±0.4 n = 6</td>
<td>( V_{1/2} = -74.3\pm1.4; ) K = 6.5±0.5; n = 9</td>
</tr>
<tr>
<td>Window current Emax (mV)</td>
<td>-35.1±0.7; n =13</td>
<td>-42.2±1^{***}; n =14</td>
</tr>
<tr>
<td>Window ( I_{in} )/peak current (%)</td>
<td>2.6±0.5; n =13</td>
<td>4.7±0.7; n =14</td>
</tr>
<tr>
<td>Recovery from inactivation, ( t_{1/2} ) (ms)</td>
<td>10.6±0.6; n = 5</td>
<td>9.9±0.9; n = 7</td>
</tr>
</tbody>
</table>

* P<0.05;  *** P<0.001

#### Table B: Electrophysiological characteristics of Nav1.5-I137V

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>p.I137V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak current at -20 mV (pA/pF)</td>
<td>-141.17±34.7; n = 6</td>
<td>-154±31.9; n = 5</td>
</tr>
<tr>
<td>Activation</td>
<td>( V_{1/2} = -28.2±0.7; ) K = 6.9±0.36; n = 6</td>
<td>( V_{1/2} = -28.7±0.4; ) K = 6.7±0.3; n = 5</td>
</tr>
<tr>
<td>Inactivation</td>
<td>( V_{1/2} = -79.4±2.3; ) K = 6.1±0.3; n = 6</td>
<td>( V_{1/2} = -77.8±3; ) K = 5.7±0.6; n = 5</td>
</tr>
</tbody>
</table>
Supplemental figure legends

Supplemental Figure 1. Experimental Effects of p.I141V Mutation on Nav1.5 Channel in HEK293 Cells

(A) Time dependence of the onset of slow inactivation was measured using the inset protocol. (B) Recovery from slow inactivation was measured using the inset protocol. (C) The distribution of peak window currents normalized to the peak current at -20mV; *p<0.05. (D) Voltage at the peak window currents; ***p<0.001.

Supplemental Figure 2. Experimental Effects of p.I137V Mutation on Nav1.5 Channel in HEK293 Cells

(A) I/V relationship from Na,1.5-WT (n=6) and p.I137V (n=5) transfected cells. (B) Steady-state activation and inactivation curves from Na,1.5-WT (n=6) and p.I137V (n=5). Parameters for the voltage-dependence steady-state of activation and steady state of inactivation are summarized in Supplemental Table 1B. (C) Sodium current activation kinetics from Na,1.5-WT (n=6) and p.I137V (n=5). (D) Fast inactivation time constants from Na,1.5-WT (n=6) and p.I137V (n=5).

Supplemental Figure 3. Sinus rate and number of PVCs per 30 seconds during 21 hour ambulatory ECG recording

Patient III-5 showed 1646 PVCs during a 21-hour ambulatory ECG recording. The upper panel shows the sinus rate and the lower panel illustrates the rate dependence of PVCs, the number of which increases upon physical exercise.
Supplemental Figure 1.

A

B

C

D

Voltage at the maximum conductance (mV)

Window current (% of peak)

Na,1.5 WT  Na,1.5 p.I141V

Na,1.5 WT  Na,1.5 p.I141V
Supplemental Figure 2.
Supplemental Figure 3.
Gain-of-Function Mutation of the SCN5A Gene Causes Exercise-Induced Polymorphic Ventricular Arrhythmias

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