Hyperpolarization-activated cyclic nucleotide-gated channel 4 gene HCN4 is a pacemaker channel that plays a key role in automaticity of sinus node in the heart, and an HCN4 mutation was reported in a patient with sinus node dysfunction. Expression of HCN4 in the heart is, however, not confined to the sinus node cells but is found in other tissues, including cells of the conduction system. On the other hand, mutations in another cardiac ion channel gene, SCN5A, also cause sinus node dysfunction as well as other cardiac arrhythmias, including long QT syndrome, Brugada syndrome, idiopathic ventricular fibrillation, and progressive cardiac conduction disturbance. These observations imply that HCN4 abnormalities may be involved in the pathogenesis of various arrhythmias, similar to the SCN5A mutations. In this study, we analyzed patients suffering from sinus node dysfunction, progressive cardiac conduction disease, and idiopathic ventricular fibrillation for mutations in HCN4. A missense mutation, D553N, was found in a patient with sinus node dysfunction who showed recurrent syncope, QT prolongation in electrocardiogram, and polymorphic ventricular tachycardia, termed de pointe. In vitro functional study of the D553N mutation showed a reduced membranous expression associated with decreased If currents because of a trafficking defect of the HCN4 channel in a dominant-negative manner. These data suggest that the loss of function of HCN4 is associated with sinus nodal dysfunction and that a consequence of pacemaker channel abnormality might underlie clinical features of QT prolongation and polymorphic ventricular tachycardia developed under certain conditions.

Heart rate is regulated by automaticity of sinus node cells. Sinus node dysfunction (SND) is a type of cardiac arrhythmia seen relatively infrequently, and a wide variety of clinical symptoms are noticed, from mild to severe, including fatigue, palpitations, anxiety, dizziness, fainting, and syncope in some cases. Physical signs of SND can also be found as inadequate heart rate at rest or in response to exercise.

Although dysfunction of the sinus node automaticity is often associated with acquired cardiac conditions such as ischemic heart disease, cardiomyopathy, congestive heart failure, or metabolic diseases, there are a few patients with idiopathic SND who do not suffer from these disease conditions. In patients with idiopathic SND, genetic abnormalities may be found in ion channel genes, similar to the cases with other inherited cardiac arrhythmias such as long QT syndrome (LQTS), Brugada syndrome and idiopathic ventricular fibrillation (IVF), and progressive cardiac conduction disturbance (1–13). One disease gene for SND is SCN5A, and its homozygous loss of function mutation leading to reduced cellular excitability predisposes to congenital sick sinus syndrome (14). The other is the HCN4 mutation (15), where a truncation mutation causes loss of exercise-induced increase of heart rate.

SCN5A encodes an α-subunit of the cardiac sodium channel carrying the current to form a rapid upstroke of action potential (16), whereas HCN4 codes for an α-subunit of hyperpolarization-activated cation channel (17). Because HCN4 is mainly expressed in sinus node cells and forms the pacemaker current (If), it is called the pacemaker channel (18). The HCN4 channel is a member of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels activated by membrane hyperpolarization. HCN channels have evolutionary conserved structure in the core transmembrane domain, cyclic nucleotide binding domain (CNBD), and linker region connecting the core domain to the CNBD (19–20). The core domain consists of six transmembrane segments containing a pore-forming P region with a glycine-tyrosine-glycine (GYG) sequence conserved in the selectivity filter among K+ channels. The HCN channels are quite diverse from the other K+ channels outside the GYG sequence, which

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FUNCTIONAL CHARACTERIZATION OF A TRAFFICKING-DEFECTIVE HCN4 MUTATION, D553N, ASSOCIATED WITH CARDIAC ARRHYTHMIA*
may account for the properties of low K⁺ selectivity and voltage dependence for activation. According to these unusual channel properties, the HCN channels conduct a net inward current carried largely by Na⁺ at diastolic potential levels.

Disturbance of the channel function in the heart may result in cardiac arrhythmias of life-threatening nature in some cases. Dysfunction of HCN channels in HCN2 or HCN4 knock-out mice causes sinus dysrhythmia because of the reduction of H current (21, 22). In addition, expression of HCN4 is not restricted to sinus node cells in mice (23), implying that there would be some other phenotypes associated with HCN4 mutations.

Here we have characterized a novel HCN4 mutation found in a patient with SND, QT prolongation, and polymorphic ventricular tachycardia, *torsade de pointes* and explored the mechanism of channel dysfunction caused by the mutation. This is the first report of a trafficking-defective HCN4 channel mutation.

**MATERIALS AND METHODS**

Expression of HCN4 in the Human Heart—Expression of HCN4 in the human heart was investigated semiquantitatively by reverse transcription and polymerase chain reaction (RT-PCR) using a human cardiovascular multiple tissue cDNA panel (Clontech). Each tissue cDNA, normalized to the expression levels of several different housekeeping genes by the manufacturer, was used as template for PCR amplification. RT-PCR was done with a pair of HCN4-specific primers of 5'-CCCGCTATTGCATACTAC-3' and 5'-GAGGCTGGTTAGGTACTGC-3' (17), and the products were subjected to electrophoresis in a 1.5% agarose gel. The density of each PCR fragment was measured after staining with ethidium bromide (ATTO Corp.).

Subjects—We analyzed 6, 3, and 16 genetically unrelated index patients with SND, progressive cardiac conduction disturbance, and IVF, respectively. Blood samples were obtained from each patient after an informed consent for gene analysis was received. These patients were first analyzed for mutations in the channel genes, including *KCNQ1*, *KCNQ2*, *SCN5A*, *KCNE1*, *KCNE2*, and parts of *RYR2* (exons 41–49 and 76–105), by single-strand conformational polymorphism (SSCP) analysis (24) of PCR products obtained by using primers reported in the literature (8, 16, 25). No patients or family relatives showed clinical signs of Andersen syndrome, such as periodic paralysis and dysmorphic features, caused by KCNJ2 mutation (9). The research protocol was approved by the Ethics Reviewing Committee of the Medical Research Institute, Tokyo Medical and Dental University.

Mutational Analysis of HCN4—We designed primers to separately amplify eight coding exons of HCN4 (Fig. 2A). Each exon was amplified by using various combinations of primers. Sequences of the primers and conditions of PCR are available upon request. The PCR products from patients were searched for sequence variations by the single-strand conformational polymorphism method and subsequent direct sequencing.

Distribution of GFP-tagged HCN4 Channel—Because human HCN4 cDNA was not available to us, we investigated functional changes caused by the D553N mutation using rabbit HCN4 cDNA (26). A mutation equivalent to the human HCN4 D553N was introduced into the wild-type rabbit cDNA (rab-WT) to obtain a rabbit HCN4 D553N (rab-Mut). GFP-tagged HCN4 constructs were made from these constructs by replacing the termination codon with a GATAAC sequence and the GFP sequence amplified with primers 5'-GATATCATGGCCAGCAGAAAAGGAGAAGA-3' and 5'-TCTAGAGCTTGTAGTAGCTCATCCA-3'.

**Fig. 2. Genetic analysis and electrocardiogram findings of patients with D553N mutation.** A, D553N mutation is indicated in the genomic structure of HCN4. B, direct sequencing profile of exon 5 from a proband patient with the D553N mutation representing a heterozygous change of AAC (Asn) from GAC (Asp). C, sequence alignment of HCN4 channels around the residue D553 of human HCN4. Dashes indicate identical residues to human HCN4 channel. D, electrocardiogram of the proband recorded at the onset of *torsade de pointes* ventricular tachycardia. E, pedigree of family with the HCN4 D553N mutation. Filled square, affected male; filled circle, affected female; open square, unaffected male; open circle, unaffected female; gray circle with a slash, female deceased without affirmation by medical records. An arrow indicates the proband patient. The corrected QT (QTc) of each affected individual is noted. All affected individuals carried the mutation.

from pcDNA3.1/NT-GFP (Invitrogen). These constructs were sequenced to ensure that no other mutation was introduced. The HCN4 constructs were transfected into COS7 cells. The amount of transfected DNA was 0.2 μg/well for each HCN4 construct (rab-WT: rab-MUT = 1:1 in the case of co-expression of rab-WT and rab-Mut) in a Lab-Tek 4 chamber mounted glass slide (Nalge Nunc International). The GFP signals from the transfected cells were examined using a confocal fluorescence microscope (Carl Zeiss AB).

Electrophysiological Analysis of HCN4 Channel—COS7 cells were co-transfected with the construct of 0.4 μg of pEGFP-C1 (Clontech) and 1.6 μg of non-GFP-tagged rabbit HCN4 constructs. Cells were removed from culture dishes 36–48 h after the transfection and placed into a chamber on the stage of an inverted microscope (Diaphot TMD; Nikon). The cells were superfused with Tyrode’s solution containing 157 mM NaCl, 4 mM KCl, 1.5 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES (pH 7.4 adjusted with NaOH). Currents were recorded by a whole cell patch clamp configuration. Glass pipettes had an inner diameter of ~1.0–1.5 μm and had resistance of 2–3 MΩ when filled with an internal solution composed of 110 mM K-gluc, 20 mM KCl, 1 mM MgCl₂, 5 mM EGTA, 5 mM MgATP, and 10 mM HEPES (pH 7.2.
Tagged HCN4 channel proteins. COS7 cells were transfected with various HCN4 constructs, and the GFP signals were detected after 36–48 h. A, GFP-tagged rab-WT. B, GFP-tagged rab-Mut. C, co-transfection of GFP-tagged rab-Mut and non-tagged rab-WT. D, co-transfection of GFP-tagged rab-WT and non-tagged rab-Mut. The transfection experiments were repeated at least three times; representative images of transfected cells are shown. More than 95% of cells transfected with mutant constructs (B, C, and D) showed cytoplasmic retention of GFP signal, whereas less than 5% of cells transfected only with GFP-tagged rab-WT (A) showed the retention. No remarkable morphological differences were noted among the cells transfected with different constructs.

RESULTS

HCN4 Expression in the Human Heart—In a semiquantitative RT-PCR analysis, an HCN4-specific PCR product was detected not only in cDNA from an atrioventricular node but ubiquitously in cardiac tissue cDNAs (Fig. 1). Density of PCR product from each tissue was measured and compared with that from the atrioventricular node. Relative ratio was as follows: total adult heart, 0.24; total fetal heart, 0.35; aorta, 0.00; apex of the left ventricle, 0.78; left atrium, 0.90; right atrium, 1.10; right auricle, 1.33; left auricle, 0.67; left ventricle, 0.29; right ventricle, 0.35; and interventricular septum, 0.68.

Mutational Analysis of HCN4—To identify arrhythmia-related gene mutations in patients with cardiac arrhythmia, we first analyzed patients with SND, progressive cardiac conduction disturbance, and IVF for mutations in the channel genes KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, and RYR2 by single-strand conformational polymorphism analysis and subsequent direct sequencing. Two SCN5A mutations were found in two IVF patients (details will be reported elsewhere), whereas no mutation was detected in the other patients; they were investigated further for the HCN4 mutation.

The analysis of HCN4 revealed a novel mutation in a patient with SND that was not found in 380 control chromosomes, implying that it was not a polymorphism and might be a disease-associated mutation. The mutation was a G to A transition in exon 5, resulting in amino acid replacement of Asp (GAC) with Asn (AAC) at codon 553, D553N (Fig. 2, A and B). This variation was located in the linker region between the core transmembrane domain and CNBD, which is an evolutionary...
conserved region among the HCN channel family (Fig. 2C). The index patient with D553N mutation was a 43-year-old woman who had suffered from syncope for the first time at age of 20. She experienced syncope again at 34 years upon hearing a ringing of the telephone. Her electrocardiogram of 24-h recordings showed severe bradycardia of 32–70 beats/min (average was 39) and a cardiac arrest for 40 s followed by polymorphic ventricular tachycardia, torsade de pointes (Fig. 2D). Her resting electrocardiogram showed flat T waves; QT interval corrected by heart rate was 0.67 s, which was shortened to 0.46 s by administration of mexiletine or pilsicainide (Na+ channel blocker). Co-segregation of D553N with the phenotype was observed in her family (Fig. 2E), and all affected individuals were heterozygous for the mutation.

**Distribution of HCN4 Channel with D553N Mutation**—To investigate a functional change caused by the mutation, we compared distribution of GFP-tagged channel proteins of wild type rabbit HCN4 (rab-WT) and mutation-introduced rabbit HCN4 D554N (rab-Mut) in transfected COS7 cells. D554N of rabbit HCN4 is equivalent to D553N of human HCN4 (Fig. 2C). Cell surface expression of rab-WT was observed as strong fluorescence signals on the plasma membrane (Fig. 3A). In clear contrast, only a weak signal of GFP-tagged rab-Mut was found on the cell surface and a large amount of GFP signal was retained intracellularly (Fig. 3B). Co-transfection of GFP-tagged rab-Mut with non-tagged rab-WT showed weak GFP signals on the cell surface and intracellular retention (Fig. 3C). Similarly, distribution of GFP signals was reduced on the cell surface in the case of co-transfection of GFP-tagged rab-WT with non-tagged rab-Mut (Fig. 3D). These observations suggested that the co-expression of mutant channels reduced the cell surface expression of normal channels in a dominant-negative manner.

**Electrophysiological Analysis of HCN4 Channel with D553N Mutation**—Functional alterations of the mutant HCN4 channel were examined by a whole cell patch clamp method applied to COS7 cells transfected with non GFP-tagged rabbit HCN4 constructs. When hyperpolarizing test pulses were applied from a holding potential of −30 mV, voltage- and time-dependent inward currents were activated at potential negative to −60 mV in cells expressing rab-WT, and tail currents were obtained at membrane potential stepped to −100 mV after various test hyperpolarizations (Fig. 4). The current amplitude for expressed rab-WT HCN4 was increased and the time course of current activation became faster with hyperpolarization of test pulses (Fig. 4A). Voltage- and time-dependent properties of inward currents recorded from transfected COS7 cells resembled H current in native sinus node cells (30). Because H contains both Ca2+-sensitive and -insensitive current components, we repeated current measurements in the presence and absence of Ca2+ to block the Ca2+-sensitive HCN4 currents. Then the subtracted current was obtained in the presence of 2 mM Ca2+ from the current in its absence (Fig. 4A). Cells transfected with rab-Mut showed decreased current amplitudes with similar time course of activation as those with rab-WT. Co-transfection of rab-WT and rab-Mut exhibited current amplitudes smaller than half sizes of rab-WT currents (Fig. 4B). The normalized tail current activation curve (I/I_max) indicates the conductance-voltage relationship (Fig. 4C), and its voltage dependence was analyzed by fitting to the Boltzmann equation to obtain various parameters of channel function. As listed in Table I, values of V1/2 and slope factor, κ, were not changed by the mutation, suggesting that voltage dependence in activation was not affected in the mutant. Time constants for current activation and deactivation were evaluated from records shown in Fig. 4, A and D. As shown in Fig. 4E, activation was faster and deactivation became slower in cells transfected with rab-Mut or rab-WT/rab-Mut than those in cells expressing rab-WT.

**DISCUSSION**

Cardiac ion channel dysfunction due to gene mutations displays a broad spectrum of clinical phenotypes, giving examples of SCN5A mutations responsible for SND, progressive cardiac conduction disturbance, Brugada syndrome, IVF, and LQTS. This is not necessarily particular to the sodium ion channel gene. As for the KCNH2 gene, knock-out mice showed the SND phenotype (27); in addition, KCNH2 mutations are well known to cause LQTS (1, 10, 12). In this study, we demonstrated an HCN4 mutation, D553N, in a patient suffering from recurrent cardiac syncope associated with severe bradycardia, prolonged QT interval, and polymorphic ventricular tachycardia. Because no mutation in the known disease-causing genes for cardiac arrhythmia was found in the patient, it was suggested that the loss of function HCN4 mutation was associated with life-threatening cardiac arrhythmia in this patient.

HCN channels are expressed preferentially in cells of the sinus node and conduction system, such as the atrioventricular node and the His bundle. However, it is not clear whether the expression of each HCN channel is confined to the cells of the conduction system. In this study, HCN4-specific RT-PCR product was found throughout the cardiac tissues, suggesting that HCN4 is expressed in cardiomyocytes of various regions at the RNA level. This is consistent with a report on the cellular localization of mouse HCN channels in the heart as assessed by in situ hybridization (23). Although HCN4 expression in the ventricular tissues is lower than that in the atrioventricular node, the distribution of the HCN4 channel in the ventricle remains to be elucidated as to its functional role in the cardiac conduction system and working myocardium.

The expressed current amplitudes of rab-Mut and rab-WT/rab-Mut for HCN4 were much decreased compared with that of rab-WT, whereas voltage-dependent properties of current activation were not affected. Although the activation kinetics of the mutant current were also changed, the overall effects were much decreased current function in this mutation. As to decreased current amplitude in the mutant, confocal microscopic analysis of GFP-tagged channel proteins suggested that the mutation caused a trafficking defect, although a complex of SCN5A mutation caused trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutation caused a trafficking defect, although a complex of SCN5A mutations linked to LQTS or Brugada syndrome (10, 28, 29).

| rab-WT (A) | rab-WT/rab-Mut (B) | rab-Mut (C) | p value of (A) vs. (B) | p value of (A) vs. (C) |
|-----------|-------------------|-------------|-----------------------|-----------------------|
| n = 8     | n = 9             | n = 9       |                       |                       |
| V1/2      | −77.98 ± 1.06     | −78.82 ± 1.20 | −78.33 ± 1.07         | 0.589                 | 0.809                 |
| κ         | 9.11 ± 1.03       | 9.25 ± 1.18 | 8.79 ± 1.04           | 0.926                 | 0.820                 |

**Table I**

Electrophysiological characteristics of COS7 cells transfected with various HCN4 constructs

Values are given as means ± S.E.
HCN4 mutation serves as a dominant-negative suppression. HCN channels play a role in slow diastolic depolarization during Phase 4 of cardiac action potential, especially in Purkinje cells and partly in sinus node cells (30, 31), modulating heart rate produced by the primary or subsidiary pacemakers. It was reported that administration of ivabradine, an HCN4 channel inhibitor, resulted in bradycardia and QT prolongation in guinea pigs (32), although the extent of inhibition depended on the dose and complete inhibition required a high dose (33). On the other hand, a frameshift mutation, P544fs, of HCN4 trafficking-defective was reported in a patient who showed a defective response of heart rate to exercise without prolongation of QT interval (15). The P544fs mutation was predicted to encode a truncated channel lacking the cytoplasmic tail including CNBD, but the membrane-spanning conformation including pore structure would not be affected. Indeed, the P544fs mutant channel was demonstrated to be functionally expressed in transfected cells (15). Because the P544fs mutant channel showed virtually little change in its function except for lack of response to CAMP, the clinical phenotype of the patient was mild and the patient would not be in danger of developing life-threatening arrhythmia. Neither recurrence of cardiac syncope nor ventricular tachycardia was reported with the P544fs mutation (15). In contrast, the index patient with the D553N mutation showed severe bradycardia and recurrent syncope, which suggests that the trafficking abnormality of D553N-defective channels may affect the heart rate at rest. Bradycardia is known to be associated with prolonged QT interval and to predispose to the development of torsade de points (34–37), as was found in the patient with the D553N mutation, but the physiological mechanism of such bradycardia-related QT prolongation remains to be elucidated.

In summary, we have demonstrated here for the first time a trafficking-defective HCN4 mutation associated with life-threatening cardiac arrhythmia. Further mutational and functional studies in other patients are required to elucidate the causative role of HCN4 mutations in various phenotypes of cardiac arrhythmia.

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Biel, M., Schneider, A., and Wahl, C. (2002) Trends Cardiovasc. Med. 12, 256–262.

Stieber, J., Herrmann, S., Feil, S., Loster, J., Feil, R., Biel, M., Hofmann, F., and Ludwig, A. (2003) Proc. Nat. Acad. Sci. U. S. A. 100, 15235–15240.

Ludwig, A., Zong, X., Stieber, J., Huhlin, R., Hofmann, F., and Biel, M. (1999) EMBO J. 18, 2321–2329.

Seifert, R., Scholten, A., Gau, R., Mincheva, A., Lichter, P., and Kaupu, U. B. (1999) Proc. Nat. Acad. Sci. U. S. A. 96, 8391–8396.

Wainger, B. J. DeGennaro, M., Santoro, B., Siegelbaum, S., and Tibbs, G. R. (2001) Nature 411, 805–810.

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Stieber, J., Herrmann, S., Feil, S., Loster, J., Feil, R., Biel, M., Hofmann, F., and Ludwig, A. (2003) Proc. Nat. Acad. Sci. U. S. A. 100, 15235–15240.

Ludwig, A., Zong, X., Stieber, J., Huhlin, R., Hofmann, F., and Biel, M. (1999) EMBO J. 18, 2321–2329.

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Biel, M., Schneider, A., and Wahl, C. (2002) Trends Cardiovasc. Med. 12, 256–262.

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Ludwig, A., Zong, X., Stieber, J., Huhlin, R., Hofmann, F., and Biel, M. (1999) EMBO J. 18, 2321–2329.

Seifert, R., Scholten, A., Gau, R., Mincheva, A., Lichter, P., and Kaupu, U. B. (1999) Proc. Nat. Acad. Sci. U. S. A. 96, 8391–8396.

Wainger, B. J. DeGennaro, M., Santoro, B., Siegelbaum, S., and Tibbs, G. R. (2001) Nature 411, 805–810.

Biel, M., Schneider, A., and Wahl, C. (2002) Trends Cardiovasc. Med. 12, 256–262.

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Seifert, R., Scholten, A., Gau, R., Mincheva, A., Lichter, P., and Kaupu, U. B. (1999) Proc. Nat. Acad. Sci. U. S. A. 96, 8391–8396.

Wainger, B. J. DeGennaro, M., Santoro, B., Siegelbaum, S., and Tibbs, G. R. (2001) Nature 411, 805–810.

Biel, M., Schneider, A., and Wahl, C. (2002) Trends Cardiovasc. Med. 12, 256–262.

Stieber, J., Herrmann, S., Feil, S., Loster, J., Feil, R., Biel, M., Hofmann, F., and Ludwig, A. (2003) Proc. Nat. Acad. Sci. U. S. A. 100, 15235–15240.

Ludwig, A., Zong, X., Stieber, J., Huhlin, R., Hofmann, F., and Biel, M. (1999) EMBO J. 18, 2321–2329.

Seifert, R., Scholten, A., Gau, R., Mincheva, A., Lichter, P., and Kaupu, U. B. (1999) Proc. Nat. Acad. Sci. U. S. A. 96, 8391–8396.

Wainger, B. J. DeGennaro, M., Santoro, B., Siegelbaum, S., and Tibbs, G. R. (2001) Nature 411, 805–810.
Functional Characterization of a Trafficking-defective \textit{HCN4} Mutation, D553N, Associated with Cardiac Arrhythmia

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