厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
分担研究報告書

QT 延長症候群、Brugada 症候群の遺伝子解析

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研究要旨　QT 延長症候群、Brugada 症候群の遺伝子解析を通じて、現時点で明らかになっていないこの疾患の病態解明の礎とする。ヒト由来試料（ゲノム DNA）を用いるため、倫理面への配慮が必要となるため、本年度は倫理審査委員会にて研究計画の承認を受け、その後国立循環器病センターよりサンプルを受領した。並行して、遺伝子解析のためのプライマー設計を行った。

A. 研究目的

QT 延長症候群、Brugada 症候群は心電図上 QT 時間の延長あるいは ST 部分の上昇を特徴とした疾患で、心室細動など血行動態を悪化させる心室性不整脈の生じる危険性が高くなり、失神発作さらには突然死の原因となる。家族性 QT 延長症候群については、これまで 6 つの原因遺伝子が単離されたが、そのうちの 4 つはカリウムチャネルをコードしている遺伝子であり（KCNQ1, KCNH2, KCNE1, KCNE2），1 つはナトリウムチャネル（SCN5A）をコードしていた。SCN5A は Brugada 症候群の原因遺伝子としても同定されている。ところが、これらの遺伝子に異常が同定されるのは患者全体の半分にも満たず、遺伝的要因の解明は不十分である。近年、DNA 多型、特に一塩基多型（SNP）が疾患の病態に関与することが示唆されており、これらの疾患における SNP の役割を解明することが必要と考えられる。上記遺伝子あるいはその他の心臓イオンチャネルもしくはチャネルの機能を修飾する役割をもつ遺伝子の SNP を網羅的に解析することにより、不整脈疾患の分子遺伝学的背景を探る端緒とするのが目的である。

B. 研究方法

病態関与が考えられる候補遺伝子領域内に存在する SNP を対象として、ケースコントロールアソシエーション解析を行う。

（倫理面への配慮）

平成 18 年 12 月に理化学研究所倫理審査委員会にて研究計画の承認を受けた。

C. 研究結果

18遺伝子を候補遺伝子として選択した。それぞれの遺伝子領域上に存在するSNP 75箇所について解析のためのプライマーをそれぞれ設計した。

D. 考察

倫理面へ十分な配慮をするため、倫理審査および研究計画の承認が慎重なものとなり、それに基づく国立循環器病センターからのサンプル受け入れが今年度後半となった。一方、遺伝子解析のための候補遺伝子選択と SNP 解析のためのプライマー設計はサンプルの到着を待たずに開始しており、今年度は 18 遺伝子領域上に存在する SNP 75 箇所について解析のためのプライマーを設計した。

E. 結論

本年度は倫理審査委員会にて研究計画の承認を受け、その後国立循環器病センターよりサンプルを受領した。並行して、来年度以降の遺伝子解析のためのプライマー設計を行った。

F. 健康危険情報

特になし

G. 研究発表

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2. 学会発表

なし

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

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分担研究報告書

Brugada 症候群の病態解明

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研究要旨　Brugada 症候群は、右側胸部誘導における特徴的な ST 上昇と夜間の心室頻拍・心室細動を特徴とし、中年男性に突然死を来たす。患者の約 15%に心臓 Na チャネル遺伝子(SCN5A)異常を認める。我々は、Brugada 症候群の病態解明を目指し、SCN5A 遺伝子スクリーニングを行い、検出された変異に関してはパッチクランプを用いた電気生理学的機能解析を行ってきた。さらに、RNAi 発現アデノウイルスを用いた経冠動脈的心筋導入によるモデル動物構築の試みや、新規原因遺伝子の探索も進めている。また、他の家族性不整脈疾患についても、病態解明のための遺伝子解析・機能解析を精力的に行っている。

A. 研究目的

Brugada 症候群において遺伝子解析、機能解析を行い、疾患の病態解明を目指す。また、基礎実験として Brugada 症候群のモデル動物を構築し、疾患の発生機序を解明する。

B. 研究方法

多施設より集まった Brugada 症候群患者の末梢血リンパ球より DNA を抽出し、エクソン毎に PCR を行う。高速液体クロマトグラフィー（DHPLC）を用いてスクリーニングし、遺伝子変異を認める検体に関してはシークエンサーにて塩基配列を同定する。また、Brugada 症候群のモデル動物を構築に関しては、RNA 干渉(RNAi)を用いた Na チャネル抑制を行う。成人ラット心に RNAi 発現アデノウイルスを経冠動脈的に導入し心電図解析、単離心筋の電流解析を行う。

（倫理面への配慮）

患者検体に関しては匿名化を行っており、京都大学医の倫理委員会にて承認済み。

C. 研究結果

Brugada症候群72症例中、新規SCN5A遺伝子異常を9症例(12.5%)に認めた。(R179X, T187I, D356N, H1200T, V1328M, G1408R, K1578fs/52, R1623X, D1741Y) 内、T187I, D356N, R1623X, K1578fs/52に関しては、徐脈性不整脈（3症例は洞不全症候群、1症例は完全房室ブロック）を合併し、培養細胞における変異チャネルを発現させると全くNa電流を認めないnon-functionalなチャネルであった。（Makiyama T, Akao M, et al. JACC 2005）

2006年、Brugada症候群のGPD1L遺伝子がBrugada症候群2型として報告され、当研究室においてもスクリーニングを行ったが、異常を認めなかった（未公表）。

Brugada症候群のモデル動物構築に関しては、既に新生児ラット心筋細胞にRNAi発現アデノウイルスを導入し、Naチャネル電流抑制を確認している。ラット心への遺伝子導入方法として低体温下に大動脈・肺動脈同時クランプ中、冠動脈よりアデノウイルス注入を行い、マーカーであるGFPの良好な導入効率を得た。この遺伝子導入後のラット心において、ランゲンドルフを用いてGFP陽性心筋の単離に成功し、現在、パッチクランプを用いた電流解析を行っている。また、テレメトリーを用いた心電図解析も進行中である。

D. 考察

Brugada 症候群における SCN5A 遺伝子異常の頻度は、12.5%（9/72）であり、これまでの報告とほぼ同じであった。Brugada 症候群 2 型の GPD1L 遺伝子スクリーニングでは異常を認めず、主な原因ではないと考えられる。RNAi を用いた

Na チャネル抑制によるモデル動物構築は全く新しい手法であり、他に類似研究はなく、新たな実験法の確立を目指すものである。

E. 結論

Brugada 症候群 72 症例中、新規 SCN5A 遺伝子異常を 9 症例(12.5%)に認めた。変異チャネルの機能解析では臨床症状に合致する Na チャネル機能低下を認めた。Brugada 症候群 2 型の GPD1L 遺伝子スクリーニングでは異常を認めなかった。Brugada 症候群のモデル動物構築に関しては、RNAi 発現アデノウイルスを用いたラット心への遺伝子導入に成功した。心筋単離にも成功し、現在電流解析、心電図解析を進めている。

F. 健康危険情報

なし。

G. 研究発表

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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

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分担研究報告書

QT 延長症候群、Brugada 症候群の遺伝子解析に関する研究

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研究要旨　これまで既に LQT1 型、LQT2 型および LQT3 型先天性 QT 延長症候群の原因遺伝子として KVLQT1, KCNH2, SCN5A, KCNE1, KCNE2, KCNJ2 などの 6 遺伝子が報告されているが、主に KVLQT1, HERG, SCN5A に変異を認める頻度が高い。また、これらの変異をスクリーニングする方法として、PCR-SSCP 法や WAVE 法が行われることが多いが、その感度や特異性においてまだ問題点がある。また、直接シークエンス法は検出率が高いがコストと時間を要する。今回、臨床症状から先天性 QT 延長症候群のタイプを予測することで直接シークエンス法での検出率を検討した。

A. 研究目的

KVLQT1, KCNH2, SCN5A, KCNE1, KCNE2, KCNJ2 の 6 個の遺伝子が遺伝性 QT 延長症候群の原因遺伝子として報告されている。多くの施設で、PCR 一本鎖構造変異多型（single-strand conformation change polymorphism，PCR-SSCP）が遺伝子変異を同定する方法として用いられているが、その検出率は高くない。今回我々は臨床タイプと直接シークエンス法で変異のスクリーニングを行いその検出率を検討した。

B. 研究方法

臨床症状に基づきタイプ分けした後に、3700 DNA Analyzer (ABI)を用いた蛍光ラベルシークエンス法で主な 3 個の遺伝子 KVLQT1, KCNH2, SCN5A の変異の検索をおこなった。

（倫理面への配慮）

遺伝子検査は倫理委員会で承認後、文書にてインフォームドコンセントを取得しおこなった。

C. 研究結果

先天性QT延長症候群の発端者144人を対象に検査を行い101名（70.1%）に変異を認めることができた。第一次スクリーニングで89人（61.8%）の変異を同定することができた。（下図）検査にかかった日数は各症例毎に平均をして2日であった。

遺伝子項目	mutaionの検出数	probandの総数	検出率
First スクリーニング			
LQT1(kvlqt1)	42	80	52
LQT2(HERG)	37	52	71
LQT3(SCN5A)	10	12	83
Second スクリーニング			
LQT1(kvlqt1)	48	80	60
LQT2(HERG)	42	52	80
LQT3(SCN5A)	11	12	92

D. 考察

臨床症状と直接シークエンス法を組み合わせた検査プロトコールは検出率が高く所要時間も比較的短期間であることから、有用な方法であると考えられた。

E. 結論

遺伝子検査を外来で行う上で従来の直接シークエンス法は有用な方法である。

F. 健康危険情報

特になし。

G. 研究発表

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2. 学会発表

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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

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2. 実用新案登録

特になし

3. その他

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III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
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Shimizu W	Chapter 50, Acquired form of Brugada syndrome	Gussak I, AntzelevitchC, Wilde A, Friedman P, Ackerman MJ, Shen WK	Electrical Diseases of the Heart: Genetics, Mechanisms, Treatment Prevention	Springer	UK	2007 (in press)	
Shimizu W, Ackerman MJ	Chapter 29, Provocative testing in inherited arrhythmias	Gussak I, AntzelevitchC, Wilde A, Friedman P, Ackerman MJ, Shen WK	Electrical Diseases of the Heart: Genetics, Mechanisms, Treatment, Prevention	Springer	UK	2007 (in press)	
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研究成果の刊行に関する一覧表

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Tan HL, Bardia A, Shimizu W, Moss AJ, Schulze-Bahr E, Noda T, Wilde AAM	Genotype-specific onset of arrhythmias in congenital longQT syndrome: Possible therapy implications	Circulation	114	2096-2103	2006
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Yokokawa M, Takaki H, Noda T, Satomi K, Suyama K, Kurita T, Kamakura S, Shimizu W	Spatial distribution of repolarization and depolarization abnormalities evaluatedby body surface potential mapping in patients with Brugada syndrome	PACE	29	1112-1121	2006
Kandori A, Miyashita T, Ogata K, Shimizu W, Yokokawa M, Kamakura S, Miyatake K, Tsukada K, Yamada S, Watanabe S, Yamaguchi I	Magnetocardiography study on ventricular depolarization-current pattern in patients with Brugada syndrome and complete right-bundle branch blocks	PACE	29	1359-1367	2006
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Does an Overlap Syndrome Really Exist Between Brugada Syndrome and Progressive Cardiac Conduction Defect (Lenegre Syndrome)?

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Editorial Comment

The Brugada syndrome is characterized by coved-type ST-segment elevation in the right precordial electrocardiographic leads (V1–V3) (a so-called Type 1 ECG) and a high incidence of sudden death due to ventricular fibrillation (VF) in patients without structural heart disease.[1,2] The prevalence of Brugada syndrome is estimated to be 5 per 10,000 inhabitants in Thailand.[3] Type 1 Brugada ECG without symptoms, i.e., asymptomatic Brugada syndrome, is commoner with a prevalence of 12 per 10,000 inhabitants in Japan.[4,5] More than 80–90% of patients affected by Brugada syndrome are males,[4,6] although males and females are expected to inherit the defective gene equally.

Experimental studies employing arterially perfused canine right ventricular wedge preparations, which have been mainly conducted by Antzelevitch and his group, have elucidated the cellular and molecular basis for typical ST-segment elevation and subsequent VF.[7] An accentuated transient outward current (I_{to})-mediated action potential (AP) notch and subsequent loss of the AP dome in the epicardial cells, but not in the endocardial cells, of the right ventricle gives rise to a transmural voltage gradient, producing coved-type ST-segment elevation in the ECG. Heterogeneous loss of the AP dome in the restricted epicardial area creates a marked epicardial dispersion of repolarization, giving rise to premature beats caused by phase 2 reentry which can precipitate nonsustained polymorphic ventricular tachycardia (VT) or VF.[8,9]

Evidence of conduction abnormality has accumulated in patients with Brugada syndrome. The original report by Brugada and Brugada published in 1992 included right bundle branch block (RBBB) pattern as one of the ECG characteristics of this syndrome.[1] Although RBBB is now believed not to be necessary for definitive diagnosis, Brugada patients have a higher incidence of complete or incomplete RBBB than the normal population. Widening of P wave and QRS duration, and prolongation of the PQ interval and HV interval, all of which represent conduction abnormality, are often observed in patients with Brugada syndrome.[10] Smits and coworkers reported greater prolongation of PQ interval in Brugada patients with *SCN5A* mutations than in those without *SCN5A* mutations.[11] Approximately 60–70% of patients with Brugada syndrome show late potentials (LPs) detected by a signal-averaged electrocardiogram.[12,13]

Several phenotypes other than Brugada syndrome have been reported to result from *SCN5A* mutations such as the LQT3 form of the congenital long QT syndrome (LQTS), cardiac conduction defect (Lenegre syndrome), atrial standstill, and atrioventricular block. Interestingly, patients with a specific *SCN5A* mutation share multiple phenotypes, thus creating a category of overlapping phenotype. Bezzina and coworkers have reported a large family affected with a specific insertion mutation, 1795insD, in which family members showed both LQT3 and the Brugada phenotype.[14] Priori et al. demonstrated that flecainide, a class IC sodium channel blocker, unmasked Brugada phenotype in 6 of 13 patients with the LQT3 syndrome.[15] Kyndt et al. described a large French family with a specific *SCN5A* missense mutation, G1406R, in which phenotypes of both the Brugada syndrome and the Lenegre syndrome were observed.[16]

Probst et al. suggest a more common association of conduction abnormalities in the *SCN5A*-related Brugada patients in this issue of *Journal of Cardiovascular Electrophysiology*.[17] They have identified intraventricular conduction defects in 59 (76%) of 78 *SCN5A* mutation carriers recruited from 16 Brugada families, while baseline spontaneous ST-segment elevation was seen only in 28 (36%) mutation carriers. They suggest that *SCN5A* Brugada syndrome-type mutation carriers exhibit various degrees of progressive cardiac conduction defects similar to the Lenegre syndrome, and therefore need clinical and ECG follow-up. These data raise the question as to whether an overlap syndrome really exists between the Brugada syndrome and progressive cardiac conduction defect (Lenegre syndrome), especially in *SCN5A* mutation carriers.

However, the relationship of the cardiac conduction defects detected in the majority of Brugada patients to the pathophysiological mechanism of VF and risk stratification in patients with Brugada syndrome seems to be clinically more important. Even though this syndrome is a monogenic inherited disorder, the Brugada syndrome typically manifests during adulthood, with a mean age of sudden death of 41 ± 15 years.[4] Cardiac conduction defects in Brugada patients, which gradually progress with age, may contribute to the pathogenesis of VF and to the late onset of first cardiac events in patients with Brugada syndrome. In other words, the mechanism of coved-type ST-segment elevation and the

J Cardiovasc Electrophysiol, Vol. 17, pp. 276-278, March 2006.

Dr. W Shimizu was supported by the Hoansha Research Foundation, Japan Research Foundation for Clinical Pharmacology, Ministry of Education, Culture, Sports, Science and Technology Leading Project for Biosimulation, and health sciences research grants (H17 – Research on Human Genome, Tissue Engineering – 009) from the Ministry of Health, Labour and Welfare, Japan.

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doi: 10.1111/j.1540-8167.2006.00406.x

first ventricular premature beat initiating VF can be explained by the accentuated I_{to}-mediated AP notch and heterogeneous loss of the AP dome in the epicardial cells as well as subsequent phase 2 reentry between the epicardial cells.[7] However, some degree of ventricular conduction abnormalities may be required to perpetuate polymorphic VT or to maintain VF.

Aiba and coworkers recently conducted a high-resolution optical mapping in an experimental Brugada model employing a canine right ventricular wedge preparation, which allowed a detailed measurement of cellular repolarization and depolarization in the epicardial and endocardial surfaces.[9] Their data suggested that the initiating phase 2 reentry-induced ventricular premature beats originated from the epicardial area with a steep gradient of ventricular repolarization time due to heterogeneous loss of the AP dome. In contrast, wave break appeared at sites of delayed epicardial conduction during the first few reentrant waves, which was closely associated with VF susceptibility, suggesting that conduction abnormalities contribute to the maintenance of VF in the Brugada condition. Kanda et al. reported that the inducibility of VF by ventricular programmed electrical stimulation was related to the severity of conduction abnormalities, such as longer QRS or HV intervals, and a higher incidence of RBBB or LPs in patients with symptomatic Brugada syndrome,[18] also indicating the role of conduction abnormalities in the maintenance of VF. Because most cardiac events occur during sleep in patients with Brugada syndrome, sustained VF or nonsustained polymorphic VT lasting more than 10 or 20 seconds is required to produce symptoms, i.e., sudden cardiac death, syncope, or nocturnal agonal respiration. The usefulness of programmed electrical stimulation to stratify risk of subsequent cardiac events is still controversial in patients with symptomatic or asymptomatic Brugada syndrome.[18-21] Brugada et al. suggested that inducibility of VT/VF is a strong indicator of subsequent cardiac events in both symptomatic and asymptomatic patients.[19] However, Priori et al.,[20] Kanda et al.,[18] and Eckardt et al.[21] failed to find an association between VF inducibility and new cardiac events or recurrence of VT/VF. If the progressive cardiac conduction defects often observed in patients with Brugada syndrome are really linked to VF maintenance, progressive conduction parameters such as QRS widening, LPs, or inducibility of VF by programmed electrical stimulation may still have a potential to predict new or subsequent cardiac events. A much larger patient population with similar patient characteristics and stimulation protocol, and a longer follow-up period are required to make a definitive conclusion regarding the predicting value of conduction parameters for new or further cardiac events.

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Common Sodium Channel Promoter Haplotype in Asian Subjects Underlies Variability in Cardiac Conduction

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Background—Reduced cardiac sodium current slows conduction and renders the heart susceptible to ventricular fibrillation. Loss of function mutations in *SCN5A*, encoding the cardiac sodium channel, are one cause of the Brugada syndrome, associated with slow conduction and a high incidence of ventricular fibrillation, especially in Asians. In this study, we tested the hypothesis that an *SCN5A* promoter polymorphism common in Asians modulates variability in cardiac conduction.

Methods and Results—Resequencing 2.8 kb of *SCN5A* promoter identified a haplotype variant consisting of 6 polymorphisms in near-complete linkage disequilibrium that occurred at an allele frequency of 22% in Asian subjects and was absent in whites and blacks. Reporter activity of this variant haplotype, designated HapB, in cardiomyocytes was reduced 62% compared with wild-type haplotype (P=0.006). The relationship between *SCN5A* promoter haplotype and PR and QRS durations, indexes of conduction velocity, was then analyzed in a cohort of 71 Japanese Brugada syndrome subjects without *SCN5A* mutations and in 102 Japanese control subjects. In both groups, PR and QRS durations were significantly longer in HapB individuals ($P \leq 0.002$) with a gene-dose effect. In addition, up to 28% and 48% of variability in PR and QRS durations, respectively, were attributable to this haplotype. The extent of QRS widening during challenge with sodium channel blockers, known to be arrhythmogenic in Brugada syndrome and other settings, was also genotype dependent (P=0.002).

Conclusions—These data demonstrate that genetically determined variable sodium channel transcription occurs in the human heart and is associated with variable conduction velocity, an important contributor to arrhythmia susceptibility. (***Circulation.* 2006;113:338-344.**)

Key Words: arrhythmia ■ conduction ■ death, sudden ■ genetics ■ ion channels

Sudden cardiac death (SCD) accounts for 20% of all mortality in Western countries.[1] One key determinant of normal excitation and conduction of the cardiac impulse is the cardiac sodium channel, responsible for rapid depolarization in most cardiomyocytes. Reduced sodium current predisposes to SCD. For example, although sodium channel blockers have been used for antiarrhythmic therapy, the Cardiac Arrhythmia Suppression Trial (CAST) showed that these agents increase the incidence of SCD.[2] Loss of function mutations in *SCN5A*, the cardiac sodium channel gene, causes ≈20% of cases of the Brugada syndrome, which is associated with a high risk of SCD.[3] Furthermore, there is evidence that such sodium channel mutations also may lead to enhanced fibrosis in myocardial tissue.[4,5]

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The overall hypothesis underlying the work presented here is that variability in regulation of sodium channel expression contributes to interindividual variability in cardiac conduction and consequently can be considered a candidate modulator of arrhythmia susceptibility, especially in the presence of other stressors such as drugs or acute myocardial ischemia.[6] As a first step in testing this hypothesis, we cloned and characterized the proximal promoter region of *SCN5A* and identified multiple cis-acting elements regulating gene expression.[7] We report here identification of an ethnic-specific, common *SCN5A* promoter variant that modulates PR and QRS durations, indexes of cardiac conduction.

Received August 3, 2005; revision received September 15, 2005; accepted October 11, 2005.

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Guest Editor for this article was Douglas P. Zipes, MD.

The online-only Data Supplement can be found at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.105.580811/DC1.

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Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/CIRCULATIONAHA.105.580811**

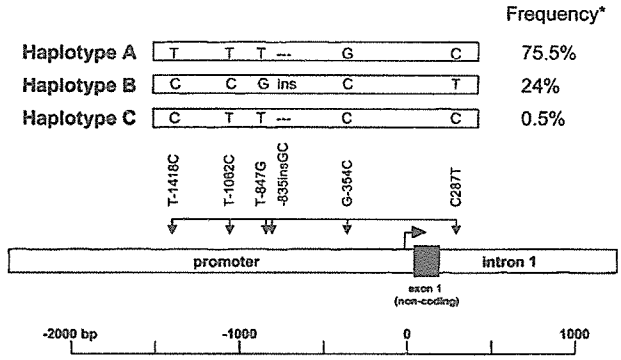

Figure 1. Haplotypes identified in the cardiac sodium channel gene (*SCN5A*) promoter. Nucleotide variations are indicated by their position relative to the major transcription initiation site (+1),[7] with the most frequent nucleotide given below and the least frequent nucleotide given above the position. *Frequency in the Japanese (control) population.

Methods

Identification of Polymorphisms

Resequencing 2.8 kb of the *SCN5A* promoter region in a single individual of Asian origin identified him as a homozygote for 6 DNA polymorphisms in the region: T-1418C, T-1062C, T-847G, −835insGC, G-354C, and C287T (Figure 1). The resequenced region encompassed positions −2190 to 613, relative to the major transcription initiation site[7] of the *SCN5A* promoter, including 2.2 kb upstream of exon 1, exon 1 (which is 173 bp and noncoding), and the proximal 439 bp of intron 1. The fragment was amplified by long and accurate polymerase chain reaction (PCR; TaKaRa kit) with primers F1 and R1 (Data Supplement Table I; see http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.105.580811/DC1). Further studies described below established that these polymorphisms were common and in near-total linkage disequilibrium, thereby identifying 2 common haplotype blocks, designated HapA and HapB. We also detected a third combination of polymorphisms, designated HapC, in <1% of subjects. In addition to the study populations, 150 white and 100 black individuals were tested for these haplotypes.

Functional Analysis

Generation of Constructs

The 2.8-kb fragment described above was amplified from genomic DNA of HapA- and HapB-homozygous individuals. These fragments were cloned into the pGEM-T Easy vector (Promega), and inserts were subsequently subcloned into the pGL3-basic vector (Promega), which contains the firefly luciferase coding sequence, to generate *SCN5A* promoter–luciferase fusion constructs for reporter assays. These constructs were designated pGL3-Hap A and pGL3-Hap B.

Reporter Activity

Reporter activity was assayed in neonatal mouse cardiomyocytes and in Chinese hamster ovary cells as described in detail previously.[7] In brief, 1 μg pGL3-Hap A or pGL3-Hap B was transfected into neonatal mouse cardiomyocytes or Chinese hamster ovary cells. In each experiment, 0.05 μg pRL-TK plasmid (Promega) encoding Renilla luciferase was cotransfected to normalize for experimental variability caused by differences in cell viability or transfection efficiency. Luminescence was measured 48 hours after transfection with the Dual-Luciferase Reporter Assay System (Promega). The pGL3-basic (promoterless) plasmid was tested in each experiment; its activity level served as the baseline.

Study Participants

Participants in the clinical study were ascertained at the National Cardiovascular Center (Osaka, Japan). All protocols (including molecular screening) were reviewed and approved by the Ethical Review Committee of the National Cardiovascular Center, and informed consent was obtained from all individuals.

The control population consisted of 102 subjects drawn from mutation-negative relatives in congenital long-QT syndrome families in which the causative mutation had been identified. Only 1 person was drawn from each family. There were 67 male and 35 female subjects ranging from 9 to 69 years of age; mean age was 40±14 years (mean±SD).

The Brugada syndrome population included 80 patients diagnosed with Brugada syndrome, defined as type 1 "coved" ST-segment elevation in V_1 through V_3 (spontaneous in 70 patients, induced by sodium channel blocker in 10 patients).[8] In all patients, physical examination, chest roentgenogram, laboratory values, echocardiography with wall motion analysis, and Doppler screening excluded structural heart disease. Aborted cardiac arrest or ventricular fibrillation (VF) was documented in 30 patients, syncope was identified in 20, and 30 were asymptomatic. All patients had previously been screened for *SCN5A* coding region mutations, and a mutation had been identified in 9 patients. The patient group included 76 male and 4 female subjects ranging from 1 to 76 years of age (mean±SD, 47±16 years).

ECG Phenotypes

ECGs were assessed by an investigator (W.S.) who was blinded to age, gender, and genetic and clinical information. Phenotypes assessed included RR interval, PR interval measured in lead II (PR_{II}), QRS interval measured in leads V_1 (QRS_{V1}) and V_6 (QRS_{V6}), ST amplitude at J point (ST_J), and ST amplitude at 80 ms after the end of the QRS (ST_{80}).

The effects of intravenous administration of sodium channel blockers on these ECG parameters were examined in 49 of 80 Brugada syndrome patients. Pilsicainide (maximum 1 mg/kg at a rate of 0.1 mg · kg^{-1} · min^{-1}) was used in 37 patients, flecainide (maximum 2 mg/kg at a rate of 0.2 mg · kg^{-1} · min^{-1}) was used in 9 patients, and disopyramide (maximum 2 mg/kg at a rate of 0.2 mg · kg^{-1} · min^{-1}) was used in 3 patients.

Genotyping

Genomic DNA was prepared from blood leukocytes. Genotyping for the T-1418C and T-1062C single nucleotide polymorphisms (SNPs) was performed by restriction fragment length polymorphism analysis after PCR amplification with *Ear*I and *Hae*III, respectively. PCR primers used to amplify the 161-bp fragment encompassing the T-1418C SNP were F2 and R2, and those used to amplify the 123-bp fragment encompassing the T-1062C SNP were F3 and R3 (Data Supplement Table II). Genotyping for the other 4 polymorphisms (T-847G, 835insGC, G-354C, and C287T) was done by DNA resequencing of both strands. PCR primers used to amplify the 638-bp fragment encompassing the T-847G, 835insGC, and G-354C polymorphisms were F4 and R4; those used to amplify the 599-bp fragment encompassing the C287T polymorphism were F5 and R5.

Statistical Analysis

Using the individual genotypes for the 6 polymorphisms, we estimated haplotype frequencies using an E-M algorithm.[9] The haplotype frequencies were used to calculate the probabilities of the haplotype pairs compatible with the genotype combinations of the multiple heterozygous patients using Bayes' theorem. Observed haplotype pair frequencies were compared with those expected under Hardy-Weinberg equilibrium in the Brugada syndrome population and control population separately with a χ^2 test. To compare haplotype pair frequencies among Brugada syndrome patients and control subjects, Fisher's exact test was used.

All quantitative phenotypes were normally distributed, and data are expressed as mean±SD. Continuous ECG phenotypes were compared between *SCN5A* mutation–negative Brugada syndrome patients, *SCN5A* mutation–positive Brugada syndrome patients, and control subjects by ANOVA adjusted for age and gender, followed by a post hoc test for pairwise comparisons. Student *t* tests were used