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SHORT COMMUNICATION

Hiroyasu Iwasa · Masahiko Kurabayashi · Ryozo Nagai
Yusuke Nakamura · Toshihiro Tanaka

Multiple single-nucleotide polymorphisms (SNPs) in the Japanese population in six candidate genes for long QT syndrome

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Abstract We report here 20 single-nucleotide polymorphisms (SNPs), including 15 novel ones, in six genes that are considered to be candidates for long QT syndrome (LQTS): 2 SNPs in *KCNB1*, 3 in *KCND3*, 3 in *KCNJ11*, 7 in *ABCC9*, 3 in *ADRB1*, and 2 in *SLC18A2*. We also examined their allelic frequencies in a Japanese sample population of LQTS-affected and nonaffected individuals. These data will be useful for genetic association studies designed to investigate acquired arrhythmias.

Key words Long QT syndrome · Single-nucleotide polymorphism · Japanese population · Cardiac potassium channel · β1-Adrenergic receptor · Vesicular monoamine transporter

Introduction

Long QT syndrome (LQTS), an arrhythmogenic disorder characterized by prolongation of the QT interval on electrocardiograms (ECGs), often causes syncope or sudden cardiac death as a result of recurrent and lethal arrhythmias. Ventricular tachycardia, torsades de pointes, and ventricular fibrillation are among the manifestations of LQTS. Five genes in which inherited mutations are responsible for this syndrome have been identified to date: *KCNQ1* (*KVLQT1*), *KCNH2* (*HERG*), *KCNE1*, *KCNE2*, and *SCN5A* (Bennett et al. 1995; Curran et al. 1995; Wang et al. 1996a,b; Splawski et al. 1997; Abbott et al. 1999). A mutant form of one of these five genes can be detected in nearly 40% of LQTS families, but the abnormalities responsible for the syndrome in the other 60% appear to be associated with other, unknown genes.

The pathogenesis of QT prolongation has been revealed only in part, and natural variations in cardiac ion channels are thought to be strong candidates for association with the disorder. For example, Kv2.1 and Kv4.3 are pore-forming subunits of cardiac potassium channels; pharmacological studies in transgenic animals have suggested that abnormalities in these channels might contribute to QT prolongation (Barry et al. 1998; Xu et al. 1999). K_{ATP} channels (Kir6.2/SUR2A) are also highly expressed in the heart (Noma 1983); they are the molecular targets of nicorandil (Hiraoka and Fan 1989), an efficient drug for treating LQTS (Fujimoto et al. 1999).

On the other hand, as sympathetic denervation of the left side of the heart can sometimes prevent sudden cardiac death in LQTS patients (Bhandari et al. 1984), it is also important to consider molecules that participate in the function of sympathetic nerves. For example, the β1-adrenergic receptor is the target molecule of β-blockers; and heterozygous deficiency of *SLC18A2*, the gene encoding vesicular monoamine transporter (VMAT2), can induce a prolonged QT interval and sudden death in knockout mice (Itokawa et al. 1999).

Here, we report our finding of multiple single-nucleotide polymorphisms (SNPs) in six candidate genes for LQTS (*KCNB1*, encoding Kv2.1; *KCND3*, encoding Kv4.3; *KCNJ11*, encoding Kir6.2; *ABCC9*, encoding SUR2A; *ADRB1*, encoding β1-adrenergic receptor; and *SLC18A2*, encoding VMAT2), along with their allelic frequencies in normal and LQTS-affected Japanese subjects.

Subjects and methods

In the course of a search for mutations in six candidate genes in Japanese LQTS patients, we identified 20 SNPs that were present in the genomic DNA of 104 LQTS

H. Iwasa · Y. Nakamura · T. Tanaka (✉)
Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
Tel. +81-3-5449-5373; Fax +81-3-5449-5406
e-mail: toshitan@ims.u-tokyo.ac.jp

H. Iwasa · M. Kurabayashi
Second Department of Internal Medicine, Gunma University School of Medicine, Gunma, Japan

R. Nagai
Department of Cardiovascular Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Table 1. PCR primers used to cover the coding regions of *KCNB1*, *KCND3*, *KCNJ11*, *ABCC9*, *ADRB1*, and *SLC18A2*

Gene	Exon[a]	Forward primer	Reverse primer	Annealing temperature (°C)	GenBank accession number
KCNB1	1.1	CTT GCC GTC GAG TGA CAG C	TCC AGG GTA CGC CAG AGT A	58	NT_002370
	1.2	GCA TGG CTC CCG CTC CAC	GCG AGT CGT GCG TGT TGC	58	NT_002370
	1.3	GCA CGC GGC TGG GCA AGC	GCT GAG CGC GCA CAT CTC	72	NT_002370
	1.4	CGC CTT CAC CTC CAT CCT CA	GGT CTC GGC CTC ACG CTT GA	58	NT_002370
	1.5	CCC GCT ACC ACC AGA AGA AAG A	CCC AGA CCA GCA GCC CCC AGA T	58	NT_002370
	2.1	ATC ATC CTG TGG GGT GGT TG	GTG GAC TGG CCG AAC TCA TC	54	NT_002370
	2.2	TCA ACA CGC TGC CTG AGC	CAC TTC TTG GGC GAG GAG	54	NT_002370
	2.3	CAT GGA GTA CCT GCT GAG G	GCT TAA GGA TGC GGA GAA T	54	NT_002370
	2.4	TCA CCG AAT CCA ACA AGA G	AGG AAG AGG ATG AGC AAG C	58	NT_002370
	2.5	CAG TCT CTG GGC TTC ACT TT	GTC TTG GGG TAG ATG TCT CC	58	NT_002370
	2.6	CCA TCA CCA TGA CTA CTG TTG	GAT TGC TTT CTC CTG TCT CTT	58	NT_002370
	2.7	TCC CCA TCA TCG TCA ATA ACT	ATT CTC CCC ATT TTT CTC AAC	58	NT_002370
	2.8	AGC ATT GAG ATG ATG GAC ATT GT	GAA GAC GAT CTG GCT TTT TCA GG	58	NT_002370
	2.9	CAA ATG GAA ATG GAC AAA GA	TGC TGA CTC CTT GGT ATT GA	58	NT_002370
	2.10	GCC AAG ACC CAA TCC CAA CC	TGA CCC CTT CTG TGC GAG TG	62	NT_002370
	2.11	AGC ACA GAG CAA ACC AAA GG	GCT GGG GAG TGA TGT CAA AG	58	NT_002370
	2.12	GCC ACC AGA TTC TCC CAC AG	GCC TCC ACA AAC CTA CCA CC	62	NT_002370
	2.13	ATC ACT CCC CAG CAA GAC TG	CAC CCT CCA TGA AGT TGA CT	62	NT_002370
	2.14	AAC TAA CAA CCC TTT GAA GC	CTG TGG TGT AGA TGG AGG AC	62	NT_002370
	2.15	CGC TGG ACT GGA GTG TGC	GCT GTC CCT CAT CAT CTG	62	NT_002370
	2.16	CAC CAG TAC ATT GAC GCA G	AAT GCT TCT GTG GAG TAA ATA C	62	NT_002370
	2.17	CTC CCC TTT ACC CAC CTC	GGC AGG GCA GTT CAG ATG	62	NT_002370
KCND3	1.1	GGC TGC CGG CCC AAG AGC	AAC CTC CGC CCA CTC ACG	65	AF166009
	1.2	GAA CAA GCG GCA GGA TGA GC	TGA GCA CGC AGC GGA ACA CC	62	AF166009
	1.3	GGC AGC ACG GAG AAG GAG TT	CGT CGT CGT AGG CAG AGA TG	62	AF166009
	1.4	GTG TTC CGC TGC GTG CTC AA	TCC TGG TTG TTC TCC GAG TC	62	AF166009
	1.5	AGT ACA AGG ACC GCA AGA GG	GAC CGA GAC AGC GAT GAA GA	62	AF166009
	1.6	CAC CAG CAC GCT GGC CCT GGT C	CTC CTC TAC CCA TGG TGA C	62	AF166009
	2.1	CTC CCT TAC ACC CTG TTT CTC TC	GGT AAA TCC GGC TAA AGT TGG AA	62	AF166010
	2.2	TTG AGT GGC GTC CTG GTC ATT GC	GTG CTC CCC CGC ATC CTT TAC AC	62	AF166010
	3	CAT TCT TTT ATT ATC TCT GCC AAT CA	ACA AGC CCA TCT ACC CCT TTA TGT TC	62	AF166011
	4	CCA CCA GCT TTT TAC TCA ATC TCT	AAA GGG TCA GGG TCA GCG ATG AAA	62	AF166011
	5	GTA CAA TCA ATG GTG TTT TTA TCT TC	AAA GGG GAG AAT CCA CAG ACT CAG AA	62	AF166011
	6.1	CCT TTT CCT ACT TCT TCT CCT TCT GC	GAC AGT GAG GGA CTT CTT GTG GAT GG	62	AF166011
	6.2	ACC CAG GCC TCA CTA CCA C	AGG GAG GGC TGC TCA CTG C	65	AF166011
	6.3	CTC AGC ACG ATC CAC ATC	GTA GAG GGG CTC ATG CAT	62	AF166011
	7.1	CCA TCA TCA ACT CAC TCT TTT C	CTA TGG AAG GAA TGT TCG TGT T	62	AF166011
	7.2	CAC AGC CAT CAT CAG CAT CC	CAT TCC CCA CTA CCC ACT CT	72	AF166011
KCNJ11	1.1	GCG AGA GGA CTC TGC AGT GA	CAC CAG GGT GT GAA CAC GT	58	NT_000564
	1.2	GAA AGG CAA CTG CAA CGT GG	TAG TGA CTT GGA CCT CAA TG	58	NT_000564
	1.3	CTG TGT CAC CAG CAT CCA GT	TGA TGA TCA TGC T G TGC GG	58	NT_000564
	1.4	TCA GCA AGC ATG CGG TGA TC	ACG CCT TCC AGG ATG ACG AT	58	NT_000564
	1.5	CTA CCA TGT CAT TGA TGC CA	GCA CTT TGA TGG TGT TGC CA	58	NT_000564
	1.6	CGT TAC TCT GTG GAC TAC TC	TGG GCT ACA TAC CAC ATG GT	58	NT_000564
ABCC9	1	TTT TAT GAA CAA GAG TTT AC	TCC CAA ATC TCA GCC ATT AG	50	AF061289
	2.1	GGA AGT GTT AAG GTT TCA GGA C	AAG AAT CCA TCT CAG GTT ATG T	50	AF061290
	2.2	TAC AAA TTC ACC ACA ACA CA	GGC ACA AGT TAC AAA GAT GT	50	AF061290
	3	ATT ATC TGG AAC CAT CAG C	GGA GAA ACA ACA AAA GTG A	50	AF061291
	4	TTC TCT TTT TGT CTT TTT CT	TGA ATC CAA GTA ACT AAA CA	50	AF061292
	5	AAT GTA GAA AAG GTT GAA AT	TGG AAG ACA GAC GCT AAA TC	58	AF061293
	6.1	ATT TTG TTA CAT CTG TTT TT	AAT ACA AAG AGG TCC AGC AA	50	AF061294
	6.2	GGC GAC CAA TTC TAC TTA GT	CCA TTG AAA TCA CCA GGA AC	50	AF061294
	7	TTT ATG GAT TTA CCT GGT TTG T	ATA GTA TTC ACA GCC TTT ACA T	50	AF061295
	8.1	TGA AAA TAA TCA TAG TGT CAA T	TGA ACA GGC ATA GCC CAT AGA T	50	AF061295
	8.2	ATA ATA AAA TCC TTA GGC TCT CTA C	CCT CTG CAG ACA AAA ATC TTA CTT A	50	AF061295
	9.1	ACC CAT TCA TCT TCT CTT TTC C	GAG CCT CTG CCA ACT TTG TAG C	50	AF061296
	9.2	AAT GGG CGT GAT TCT GCT CTA T	TGT TAC TTA CAA GGC ACT TTT C	50	AF061296
	10	GGA TGC AGC TGG TAT TAT TTT TAC T	AAA GTC GCC TGT TCT TAG AGC AAC C	50	AF061297
	11	TAC TCT TTG TCC TTC CTG TCT TCA A	CAC TGA CTT GGT TTC ATT TCT AAA A	55	AF061298
	12	TTT TTC CAG CTT TCT CTA AA	AAA ATG CCA GAC ACT TCA GT	55	AF061299
	13	TCT TAT TCT GTG TTT ATT GTT TTT	CTG CTG AGA CTG TCC TCT ATT CAC	55	AF061300
	14.1	AAG CCT CAA CTT GTG TTC TTT TCC AT	TTT ATT GCA ATG TCC TCT GTT TCT GC	50	AF061301
	14.2	CTA TGA GCA ATC AAC ACG GCG TCT ACG	TGG GAC ACT TTC ACA GAA CTT CAC CAT	50	AF061301
	15	GGT CTC CCA AAG TGC TGT GAT TAC A	CTT GGA AAA CTA TGG TTA CGG TCA T	55	AF061302
	16	TCT GTA GTT ATC CTT TGT TT	TAT GAC ATA GCA ATG GAA GC	55	AF061303
	17	GTG TTT ATT GTA TCT CTT ACC TGA CG	TCT GAG GAA GGA AGT TAT TCT TAT TA	55	AF061304
	18	GTA CCT TTG CAT AAT CCT GGT GTT TT	AAA GAG TTG CCA TTT TCG GTT CTA AA	55	AF061305
	19	ATG GAC AAT GTA TTA CTT GAG AT	GAT TAA AGA CAA CCA GAA GAC TT	50	AF061306
	20	AAT GGG TAT TTG GGG GTG TCT CT	TTC CTT GAG TTA CTT GAC TTA CA	55	AF061307
	21	TCC ATC TTT CCT CCA TTT TA	GAA CTA CCT TTA TTT CAG AA	55	AF061308
	22	CTT TTC GTC ACA TTT CTG GGA ATA	CTC TTT TGA ACA TTG GGC TAT GAA	55	AF061308
	23	TAA GTC ACA ACC TAT TGG TGT TAG C	TCG CAT CCT GTT ATC CCA TTA GAA T	55	AF061309
	24.1	CTG AAG CTT AAA CAA AAG TT	CAG GAT GAG CAG GAA GAA TC	50	AF061310
	24.2	AAA TGC CAT GGA AAA CCT G	AAA ACA AAA CCG AAC CAA T	50	AF061310
	25	TAT GTG CTT TTT GTG TTG GT	ATG TGT GGA TGA GAA ATA AC	50	AF061311

Table 1. Continued

Gene	Exon[a]	Forward primer	Reverse primer	Annealing temperature (°C)	GenBank accession number
	26	TTG ATC ATA TAC TTC CAT CTG TT	ATT TAG CTT ACA TTG TGA AAC TA	55	AF061311
	27	GCT AAT CAT ATT TTC CTT TT	AGG GCT GAC TGT AGA TAA GC	55	AF061312
	28	TAG TTT TTG AGT CTG GAT GC	TTT GTT GAT GAT GTT ATT GC	55	AF061313
	29	GTT TCT TAT TAC CTC TCC TG	TTA CAA CTG TCT GCC TCT TT	55	AF061314
	30	CTT TTA ACA TTT TCT CTT GCT TT	ATT CAA TTC CCA TTT ATG AGT GA	55	AF061315
	31	TGG AGT GGT GAA CAT CGT GT	GTC TTC TCT ATT TGG TTT CC	55	AF061316
	31	TGG AGT GGT GAA CAT CGT GT	GTC TTC TCT ATT TGG TTT CC	50	AF061316
	32	CTG CCT ATT ATC TGT CTA TTT C	CTT GGC TGG GAA GTA TGA AGA G	50	AF061317
	33	TCT CTT TGA GTG ACA CCT GTT	TTA AAG GAA AGC ACC AAG TGG	50	AF061318
	34	ACC CCT AAC TAA GAA ATA ACT TT	AGG CAT ACA GGT GCT GCT AAA TA	50	AF061319
	35.1	CAA AAC AGA ATG GCA CAT ACA T	GCA TAA AAC GTA AGA TAG ACA T	50	AF061320
	35.2	ACC TAA TTA TTC CCT ACA GAT TT	GCA TAA AAC GTA AGA TAG ACA TA	50	AF061320
	36	CTT GCT TCT TTC TCT GAT TTA G	TTT TCA TGC ACA TTT CTC CAA T	50	AF061321
	37	TGA CAA AGT AAT CCC GAG AAA A	GAG GTA TAC CAA CTC CGT CTT C	50	AF061322
	38	CAT TTT TCC TTC TGC TGA TTC T	TTA GCC ATG CCT TAC TGA GAG G	50	AF061323
ADRB1	1.1	CGG GCT TCT GGG GTG TTC C	GAC GCG GGC ACC AGC AGC	65–62TD[b]	NM000684
	1.2	CCG CAC CGC TCC CCG ACG	GCC CGC CAC GAT GAG CAG CAC	70–67TD[b]	NM000684
	1.3	ATG GGT CTG CTG ATG GCG	GTA CTC CCA GCG GCC CC	65–62TD[b]	NM000684
	1.4	CCG TTC GGG GCC ACC ATC GT	GCG CGT CAG CAG GCT CTG GTA G	72–69TD[b]	NM000684
	1.5	CGC TAC CTC GCC ATC ACC TCG C	CGG TTG GTG ACG AAG TCG CAG C	71–68TD[b]	NM000684
	1.6	CGC CGC TGC TAC AAC GAC CC	TGG GCC GCC GAG GAA ACG	70–67TD[b]	NM000684
	1.7	GCC CAG AAG CAG GTG AAG AAG ATC G	GCC GCC GCT TAC CCG CAC	65–62TD[b]	NM000684
	1.8	CTC GCC CTC GCC CGT CCC	GCA GCC AGC AGA GCG TGA AGA C	72[c]	NM000684
	1.9	CGC TCA AGA CGC TGG GCA TCA T	CGG AAG TCG GGG CTG CGG	65–62TD[b]	NM000684
	1.10	CAA CTC GGC CTT CAA CCC CAT C	ATC GTC GTC GTC GTC GTC CG	68–65TD[b]	NM000684
	1.11	GAC CCC CGC CAT CGC CCG	CGG GGC GGC ACG GCT CG	72–69TD[b]	NM000684
	1.12	GGC GGC GGA CAG CGA CTC	TGA TTC AGA CGA GGA TTG TGG GC	68–65TD[b]	NM000684
SLC18A2	1	TAA ACT GAG CGG CGG CGG	ACC GGG GCC GTG TTA CCT C	62	NT_000545
	2	CCT GGG GGA CGG GAG AAT G	CAG CCA GCG GAC CAG CG	62	NT_000545
	3.1	GTG ATC CAC CTG CCT CAG C	TCT CAT GCT TAA TGC TGT ACA GAT	62	NT_000545
	3.2	ACC ACC TTG CCA TTC TGC TCT TAT C	TCA GGT CTC TGG TAG CAT TCC CG	62	NT_000545
	3.3	GCC TCC ATC TCA GAC AGC	AGG AGG TCT TTG TCT TCA CTG	64	NT_000545
	4	AAG GGT AGC CAG GCG AAA ATC A	TCA ATG CCC ACC CTG ATG AGA C	56	NT_000545
	5	ACG GTT GTG TCC CAG CAG C	GCC CCT CAC GAA CTC ACT CA	64	NT_000545
	6	GGG AAG GCG AGT CAC GC	GGA CTG CCG CTG TCC AGC	58	NT_000545
	7	CTA GTA CAG GGA GAG GGC ATG T	AAG GTC ATG GCA TGG CG	62	NT_000545
	8	TGC TTG GGC CAC ACC TGA TT	CCC TCA TTC CCA GCC GCT	58	NT_000545
	9	GAG GTA AGC GGC TGG GAA TGA G	CCT CGC ATT CCC CTG ACT CA	58	NT_000545
	10	TGG AAA TGA GAG AGG AGG CAG	AGA AGA GCC CAC CCC AGT C	62	NT_000545
	11	TTA CTT TTT CTA ACA TAT GAC TGA T	TGA GTG TTG TTT TCA TAT CAT CT	58	NT_000545
	12	TTG GAC TAG AAA AAA ATC AAA TGA TC	CAC AGA TTG TTC CCA CTT GTC G	62	NT_000545
	13	GAG TTG GAT CTC TTG TTA TTT TAG CA	TAA ATG TAG GCA CAA GGT TCG T	58	NT_000545
	14	CTA TAC ACT GTG TTC CCT GAC TGT C	GGT TCT TAT GAA AAG CCA ATG TC	62	NT_000545
	15	GCT TTA TCT AAT TCT CTG TTT CC	TTA TCA AGA AAT GCT CAC TGC	62	NT_000545
	16.1	AGA AGT TAA TAT ACT TGC ACT TTG C	CTG TTT TAT ACA ATT AAA CAC TTT GAT G	58	NT_000545
	16.2	ATG AAG AAT CTG AAA GTG ACT GAG	AAG GCT GTT GGC AAT CG	62	NT_000545

SSCP, Single-strand conformation polymorphism
[a] Exons followed by dots and sequential numbers were subdivided for SSCP analysis
[b] TD, indicates touch-down polymerase chain reaction (PCR). [c] Shuttle PCR was used

patients and 150 normal controls. Our screening method was described previously (Itoh et al. 1998). In brief, we prepared genomic DNA from blood samples according to standard protocols, after obtaining written informed consent from each participant. All exons of each gene being scrutinized (*KCNB1*, *KCND3*, *KCNJ11*, *ABCC9*, *ADRB1*, and *SLC18A2*), as well as flanking intronic sequences, were amplified by newly designed polymerase chain reaction (PCR) primers (Table 1) and subjected to single-strand conformation polymorphism (SSCP) analysis. Fragments presenting aberrant conformers were sequenced with ABI 3700 instruments (Applied Biosystems, Foster City, CA, USA).

To investigate the allelic frequencies for each SNP in our normal control population, we hybridized allele-specific oligonucleotides to DNA from 150 normal, unrelated individuals in the manner described by Saiki et al. (1986). An oligonucleotide specific for each allele was synthesized to discriminate between alleles at each polymorphic site.

Results and discussion

In all, we confirmed 20 SNPs (2 in *KCNB1*, 3 in *KCND3*, 3 in *KCNJ11*, 7 in *ABCC9*, 3 in *ADRB1*, and 2 in *SLC18A2*), and we examined the frequency of each allele in the Japanese population. Table 2 summarizes the results.

Six variations were detected in one of the LQTS patients (Table 2). Because none of these would change an amino acid, and because none would seem to lead to splicing abnormalities, judging from their surrounding sequences, at

Table 2. SNPs of six candidate genes for long QT syndrome

Gene	Nucleotide change[a]	Amino acid change	Region	Frequency of minor allele	Number of chromosomes examined	Previous report (if any)
KCNB1	1071C > T	D357D	Exon 2	0.01	100	
	768G > A	K256K	Exon 2	Rare[b]	188	
KCND3	375G > A	P125P	Exon 1	0.22	100	
	1106 + 15G > A	Intronic variant	Intron 1	Rare[b]	188	
	1269 + 15C > A	Intronic variant	Intron 2	0.22	100	
KCNJ11	67G > A	E23K	Exon 1	0.38	100	Hani et al. 1998; Sakura et al. 1996
	570T > C	A190A	Exon 1	0.35	100	Sakura et al. 1996
	1009A > G	I337V	Exon 1	0.38	100	Hani et al. 1998; Sakura et al. 1996
ABCC9	142 + 47C > T	Intronic variant	Intron 1	Rare[b]	188	
	285 − 22G > T	Intronic variant	Intron 2	0.03	100	
	1012 − 59T > C	Intronic variant	Intron 6	0.26	100	
	1660 − 65C > T	Intronic variant	Intron 11	0.26	100	
	2397 + 58A > T	Intronic variant	Intron 20	Rare[b]	188	
	3561 + 4C > G	Intronic variant	Intron 29	Rare[b]	188	
	4488T > C	F1496F	Exon 36	Rare[b]	188	
ADRB1	145A > G	S49G	Exon 1	0.17	100	Maqbool et al. 1999; Podlowski et al. 2000
	1071C > T	R357R	Exon 1	0.02	100	
	1165C > G	R389G	Exon 1	0.15	100	Maqbool et al. 1999; Tesson et al. 1999
SLC18A2	813 + 15T > C	Intronic variant	Intron 6	0.30	100	
	688A > T	M230L	Exon 6	0.070	300	

SNP, Single-nucleotide polymorphism
[a] Nucleotide numbering starts from ATG start codon
[b] Indicates that the allele was not identified in normal controls

present, we are considering all six to be rare polymorphisms.

Of the 20 different SNPs detected in this study, 5 had been reported already, and their allelic frequencies had been determined in Caucasian populations (Sakura et al. 1996; Hani et al. 1998; Maqbool et al. 1999; Tesson et al. 1999; Podlowski et al. 2000). We found no apparent differences in allelic frequencies for these five SNPs between Caucasian and Japanese populations.

We believe the data reported here will provide useful information for association studies designed to identify genes related to nonfamilial arrhythmias.

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