SHORT COMMUNICATION

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Twenty single-nucleotide polymorphisms in four genes encoding cardiac ion channels

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Abstract We here report 20 novel single-nucleotide polymorphisms in four genes that are potentially involved in the excitement of cardiomyocytes: 1 in *KCNA5* (encoding Kv1.5), 5 in *KCNAB1* (encoding Kvβ1.3), 5 in *KCNIP2* (encoding KChIP2), and 9 in *CACNA1C* (encoding a cardiac L-type voltage-dependent calcium ion channel, dihydropyridine receptor). We also examined their allelic frequencies in Japanese individuals. These data will be useful for genetic association studies designed to investigate secondary long QT syndrome or other circulatory disorders.

Key words Long QT syndrome · Single-nucleotide polymorphism · Japanese population · Voltage-gated potassium ion channel · KChIP · L-type voltage-dependent calcium ion channel

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 arrhythmia. Five genes in which inherited mutations are responsible for this syndrome have been identified to date: *KCNA9* (*KVLQT1*, *KCNQ1*), *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).

Since mutations in any of these five genes can be detected in nearly 40% of LQTS families, this syndrome in the remaining families is likely to be caused by other unknown genes.

Voltage-gated ion channels play important roles in the excitement of cardiomyocytes, and all the genes responsible for LQTS identified so far belong to this group. Although many channel proteins have been identified at present, only a few have been reported to be involved in cardiac excitement. For example, in mice, decreased expression of Kv1.5, encoded by *KCNA5*, prolongs QT-intervals of ECGs (London et al. 1998), and hKvβ1.3, encoded by alternative splicing of *KCNAB1*, is expressed in human ventricle and alters the functional properties of hKv1.5 (England et al. 1995). A novel class of auxiliary proteins for Kv4α subunits, K-channel interacting proteins (KChIPs), modulates the transient inward potassium current (I_{to}) (An et al. 2000). KChIP2, encoded by *KCNIP2*, is expressed with a steep gradient across the human ventricle and might be involved in the transmural gradient of I_{to} in the free ventricular wall (Rosati et al. 2001), and KChIP2^{-/-} mice are susceptible to cardiac arrhythmias (Kuo et al. 2001). An inward calcium ion current generated by an L-type voltage-dependent calcium ion channel, encoded by *CACNA1C*, is implicated in early afterdepolarizations (January and Riddle 1989), oscillations in cell membrane voltage thought to initiate arrhythmias caused by long-QT syndrome.

Here we report single-nucleotide polymorphisms (SNPs) and other genetic variations found in four genes encoding cardiac ion channels along with their allelic frequencies among normal and LQTS-affected Japanese subjects.

Subjects and methods

In the course of a search for mutations in these candidate genes in 73 unrelated Japanese LQTS patients, we identified genetic variations. Our screening method was described previously (Itoh et al. 1998). In brief, we prepared genomic DNA from blood samples according to standard protocols,

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Table 1. Polymerase chain reaction (PCR) primers used to cover the coding region of *KCNA5*, *KCNIP2*, *KCNAB1*, and *CACNA1C*

Gene	Exon ^a	Forward primer	Reverse primer	Annealing temperature	Referenced cDNA sequence	Referenced genome sequence
<i>KCNA5</i>	1.1	ACC CCA GTC TCC CCA GAG	ACG ATG AAG AAG GGG TCG	58	NM_002234	NT_024413
	1.2	AGA CCC TGC CTG AGT TCA G	CCA TTC CCT ACT CCA CTG C	62	NM_002234	NT_024413
	1	GCT CAC CTG CTG CCT AGT G	GCC TCC TGT CAC ATA CGC	58	AY026329	NT_030094
<i>KCNIP2</i>	2	GTC CCA TTA AAC CAA GTG C	CCC TCC ACA CAC ATG CAG	58	AY026330	NT_030094
	3	CGC TGA TAG ACC TCG AAG ATG	CCC CAG AAC CTC CAG CTA G	58	AY026331	NT_030094
	4,5	CCC TCC CAT CAC ATG AGC	TTT GGC CTG GAG ATC CTG	58	AY026331	NT_030094
	6,7	GAG TTC AGG GCC AGG ATC	CCT CCA GGC TAC CCA CTC	58	AY026331	NT_030094
	8	CAG GTA CAC GTA CCC TGC AC	TGC CCA GAG AGA CCT GAG	62	AY026331	NT_030094
	9	TCC CCG TCT GTG ACT CAG	GGA AGC ACC ATA GCA GGA G	58	AY026331	NT_030094
<i>KCNAB1</i>	1	TTA AAG TTA AGC ACC GTG	GCA ATC AGC AGT ACC TGT AT	58	England et al. 1995	England et al. 1995 ^c
	2	GAC AAT ATG AGA CTG AGA AGC TG	TGG GAG AGG GTC ATG ATG	58	England et al. 1995	NT_005553
	3	GGC TTC CAA TAG TAT GTA CAG TG	CCA AAT TGT TTC ATT CTC TGA C	58	England et al. 1995	NT_005553
	4	GAA TGT TGT CAA ACC AGC TC	TGC CCC TCA CAT AGA CAT G	58	England et al. 1995	NT_005553
	5	ACC AAA CTG CAA AAT GAC TG	AGA AGC CAT GAA ATT GTC AC	58	England et al. 1995	NT_005553
	6	ACA TGG GCT TGC TGT CAG	ACA AAA AAC CCT CTC TCT AAG C	58	England et al. 1995	NT_005553
	7	ACA GAG TGT ACG TGG CTG AG	TTC TGA ATT TGT TTG GTT GAT AC	58	England et al. 1995	NT_005553
	8	GTG TAT ATT TTC CTG TGA GAG TG	ACA AAA GTG AAA TGA ACC TG	58	England et al. 1995	NT_005553
	9	AAT GGA AAA TGG AAT GTC AG	TGT ATT CTG AAC GGA TGA GC	58	England et al. 1995	NT_005553
	10	CTT AGT GAA ATC GGT GGT GAC	TGC TTG GCT TTC ATC AGT G	58	England et al. 1995	NT_005553
	11	TGC ACT CCC AAG AAC TGA C	TTC TTC CCT CCT TCC TAC TC	58	England et al. 1995	NT_005553
	12	AAC TTC TGT GCA GGC TGA TC	TCA TCA AAC GTC AAA TAA CTC AC	58	England et al. 1995	NT_005553
	13	ATT GTC CAG CGC CTC TAT G	AAT GAG CTT TGG GAA TAC TGC	58	England et al. 1995	NT_005553
	14	ATT ATA TTT CTG TTG GAT AGA GCT G	CCA AAT GCC TGA AGG TAG TAC	58	England et al. 1995	NT_005553
<i>CACNA1C</i> ^b	1	TCC TCT TCG TGG CTG CTC	GTG GAA AAG GCA GGG AGC	58	NM_000719	AC005342
	2	GAA GTG CCC CTG TTT TCT ATC TAG	GCA GGT CCC AGG AGC ATG	58	NM_000719	AC006051.1
	3	GGC ATT AAC TTC CTT GAC TC	TGA AAA AGC AGA GAC TTG AG	58	NM_000719	AC006051.1
	4	ATT TTT CTC TCT TTT CTA TTT CTG	AAG GGA AAG AAA CAG ACA GTG	58	NM_000719	NT_000719
	5	AAA ACC CAG TCC TGA CAG TC	GTA CAC AAG AAC GGG CTT C	58	NM_000719	NT_000719
	6	CTT GGT TCA GTG AGT GGC TC	GGG AGC GAT AGA GGG CAG	58	NM_000719	NT_000719
	7	CCA TCC CAT CAA CCT CAT C	CTC GTT AGG GTG AAA AGC TC	58	NM_000719	NT_000719
	8	GGT CCC CTT CGG TCA CAG	GCC AAT GGG TCA GAA TCT G	58	NM_000719	NT_000719
	9	GGA GCT GTC TGT GGA AAG TAG	GTG GTT AGG GTG AAA AGC TC	58	NM_000719	NT_000719
	10	GCT CTG CTT CCC TTT GAC TC	GCT TTT CCA CCC ATG CTC	58	NM_000719	NT_000719
	11	CCT CAG TCT CCA CAT TCA AAT AC	TGG ATT ATT TGT CTG ATC TGA GC	58	NM_000719	NT_000719
	12	GAG GAG TTC AGG GGC ATC	GGT GTC TCC AGC ACA GCA C	58	NM_000719	NT_000719
	13	CGG GCT TCT GAC CAC TG	CCA GGG CTG ATC TAG GAT G	58	NM_000719	NT_000719
	14	CGG GCT TCT GAC CAC TG	CCA GGG CTG ATC TAG GAT G	58	NM_000719	NT_000719

Table 1. Continued

Gene	Exon ^a	Forward primer	Reverse primer	Annealing temperature	Referenced cDNA sequence	Referenced genome sequence
	15	TCT AAG GAC AGT TCA ATA GCT CAG	CCA GAT TAC GGC GAC AGC	58	NM_000719	NT_000719
	16	GAA CAC TGT TGG GGT CTG AC	CCC TAC CAG AGT GAT GCT TAG	58	NM_000719	NT_000719
	17	TTT TTT CTG CTG CTG ACT G	CTC ACA GCT CCA GGA AGG	58	NM_000719	NT_000719
	18	CCT TCT CCC CTG TGA CTG	ATC CCA GGT TAG GCA GTG	58	NM_000719	NT_000719
	19	TGG AGT TAT TTA GAA TGG TGC TG	CAC CTT CCC CAC CAT TGC	58	NM_000719	NT_000719
	20	TTG ACT TGT TCC TCC TCC TGT C	GTG CAG AAG ACA CAG GCG	58	NM_000719	NT_000719
	21	ATC CCA TCC CCA CCC TGT	GGA GGA GAA GAA GGG GAT G	55	Soldatov 1992	NT_000719
	22	GAG CCA CTA ATC CAA TTA TGC T	GAT GCA TTG CCC AGG TTG	58	NM_000719	NT_000719
	23	CTG GTG TTC CTT TGT CCC TC	CTA GCT GCA TGG CCC ATC	58	NM_000719	NT_000719
	24	CAG GGT TAC AGG CAA GCT C	CAT GCA GTG GGA CCA GTG	58	NM_000719	NT_000719
	25	GCA TCT CTT GAA GCC ACG TC	CCA CAA CGG AGC CCA GAC	58	NM_000719	NT_000719
	26	GAT TAC TGA ACA TCT CTG ATA CTC TG	CTG CCC TCC CAC CTC TAC	58	NM_000719	NT_000719
	27	GTG AAG GAA GAT GGG AGA TC	GGC CTT GCT TTG CAT ACT C	58	NM_000719	NT_000719
	28	CAA GGT CAC ACA GCC AGT AAG	AAG ACT GAC AGC CCA CTC TG	58	NM_000719	NT_000719
	29	TAA CCC CAC TCT CCC CAT C	ATG GCT GTG CTC CTC CTG	58	NM_000719	NT_000719
	30	AGC TCC TCC CTT CTC CTG	GGG AAT CAA AGG TCT TTC TCA G	58	NM_000719	NT_000719
	31	CTG GGG AGG GCA GAG TAA TAC	TGC CTG CCC TCC CTT ATG	58	Soldatov 1992	NT_000719
	32	CTC TCC CCG GCT GCT CTG	GGT TAG GGA CAG ACG CCA TG	58	NM_000719	NT_000719
	33	GTG GGT CCT TGA TTG ACA TGA G	CCA CAT GCA GTG AGA GCC AG	58	NM_000719	NT_000719
	34	GCC TGG GCA CAG TCT AGC TC	CCA CAC ACA GGA AGG AGC AG	58	NM_000719	NT_000719
	35	TCA TGG GAG TCT CCT GCA C	GGG AACTGG GGA GAG ACA	58	NM_000719	NT_000719
	36	GCA TGG GAA GAC TGT TCA GC	GGT TGC TCT GTG GAA AGT TAT C	58	NM_000719	NT_000719
	37	TGA ACG TGG CTC TCC CTC	GCT GCT GGC TGT TGA GTT C	58	NM_000719	NT_000719
	38	ATT GTA CTG TTC CCC ACA GAT C	TCT CAG CTT GGG GAA TCA G	58	NM_000719	NT_000719
	39	CTC TGA TGC CCT GTC CCT C	CTC CCA TGA ACC CCG TTA TC	58	NM_000719	NT_000719
	40	CTT TGC TTC TCT TCG CCT G	CTG AGT GTC TCT CCC AGT GC	58	NM_000719	NT_000719
	41	CAC CAA GGG GCT GAG GAT C	GTG CGG GAG TCC AGG GAG	58	NM_000719	NT_000719
	42	TCC CCT CTC CAT ACG TCT C	GGA CCT TCC TGG AGT GTG	58	NM_000719	NT_000719
	43	TGG AGG AAA GGG AGC GTG	GGT CGA GAG TGC GCC ATG	58	NM_000719	NT_000719
	44	CCC ACC CCT CCT TCT TGC	CTG TCT CCT GAG GGT TGC AG	58	NM_000719	NT_000719
	45	TAA AGA TGA CCT GAC CCT GTC	AAA GGC ACA GAG AGG AAG C	58	Soldatov 1992	NT_000719
	46	CAG TTT CTG ATG TTT TTC TTC AT	GGG TCA GCC TCA GCA GAG	58	NM_000719	NT_000719
	47	GGA ACA AGC CCC ATG AGC	ACA TCC AGA GAG CGA GCG	58	NM_000719	NT_000719
	48	ATG GTG GCT CTC TGG CTG	GCC TGT CCA AAA GTG TGA G	58	NM_000719	NT_000719
	49	TCG GCC ACT CCT ATT AAC TCA C	CCT GCC CAT CTG CGA GTC	58	NM_000719	NT_000719
	50	TTC CTT TGG TTC TTC ATG GC	TAA AAA AAA GCC CGC ATC TG	58	NM_000719	NT_000719

^aExons followed by dots and sequential numbers were subdivided for analysis^bExon numbering and position of the splicing junctions of *CACNL1A1* refer to intron-exon boundary sequences of *CACNL1A1* isoform 1 (Soldatov 1994)^cPCR primers for this exon were designed based on the sequence described in the reference

Table 2. Genetic variations of four candidate genes for long QT syndrome

Gene	Nucleotide change ^a	Amino acid change	Regions	Frequency of minor allele ^b		Number of chromosomes examined	
				LQTS	Control	LQTS	Control
<i>KCNA5</i>	1149C>T	G383G	Exon 1	0.007	0.005	146	192
<i>KCNAB1</i>	348>T	I127I	Exon 4	0.007	Rare	146	192
	357+77G>A	Intronic variant	Intron 4	0.007	Rare	146	192
	525-63T>C	Intronic variant	Intron 6	0.01	0.02	146	188
	525-50A>G	Intronic variant	Intron 6	0.01	0.02	146	188
	658-57G>A	Intronic variant	Intron 8	0.06	0.05	146	192
<i>KCNIP2</i>	73+91C>A	Intronic variant	Intron 1	0.10	0.23	146	100
	294+98C>T	Intronic variant	Intron 3	0.08	0.11	146	192
	649-44T>C	Intronic variant	Intron 7	0.51	0.49	146	100
	759+90C>A		3'UTR	0.007	0.01	146	192
	759+229C>G		3'UTR	0.007	Rare	146	192
<i>CACNA1C</i>	522G>A	A174A	Exon 4	0.01	0.01	146	192
	966C>T	H322H	Exon 7	0.007	0.01	146	192
	1216+11G>A	Intronic variant	Intron 8	0.09	0.10	146	192
	2437C>T	R813W	Exon 17	0.38	0.38	146	96
	2574>G>A	S858S	Exon 19	0.01	0.01	146	96
	3846C>T	F1282F	Exon 30	0.45	0.46	146	96
	5363A>G	Q1788R	Exon 44	0.39	0.33	146	96
	5603C>T	P1868L	Exon 45	0.48	0.50	146	96
	5605A>G	M1869V	Exon 45	0.47	0.49	146	96

3'UTR, 3' untranslated region; LQTS, long QT syndrome

^aNucleotide numbering starts from the ATG start codon

^bRare indicates not identified in normal controls

after obtaining informed consent from each participant. All exons of each gene being scrutinized, as well as flanking intronic sequences, were amplified by newly designed polymerase chain reaction (PCR) primers to cover the coding region of each gene (Table 1) and screened by single-strand conformation polymorphism (SSCP) analysis, and aberrant conformers were directly sequenced with ABI 3700 instruments (Applied Biosystems, Foster City, CA, USA). Some exons were screened by direct sequencing analysis, depending on the length of the PCR fragment.

To investigate the allelic frequencies for each polymorphism in our control population, we distinguished allele-specific sequences either by using PCR-RFLP (restriction fragment length polymorphism) or by hybridizing allele-specific oligonucleotides to DNA from 48 to 96 normal, unrelated individuals in the manner described by Saiki et al. (1986).

Results and discussion

In total, we confirmed 20 novel SNPs (1 in *KCNA5*, 5 in *KCNAB1*, 5 in *KCNIP2*, and 9 in *CACNA1C*), and examined the frequency of each allele in the Japanese population, as summarized in Table 2. Three genetic variations were not detected in any of the 192 chromosomes of the normal controls examined. However, they did not change the amino acid sequences and were unlikely to affect splicing, and we consider them merely to be rare genetic variations.

We believe that the data reported here will provide useful information for association studies designed to identify genes related to nonfamilial arrhythmias, for instance, drug-induced arrhythmia. In addition, variations in *CACNA1C*, which encodes dihydropyridine receptor, the target molecule of calcium antagonists, might be helpful in genetic research for other circulatory diseases, for example, hypertension.

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