National Institute of Health Sciences, the Pharma SNP Consortium, and the Tokyo Women's Medical University approved this study.

Genetic Analysis of SCN5A

Genomic and cDNA sequences of *SCN5A* were obtained from GenBank (GenBank accession numbers NT`022517.16 and NM_198056.1, respectively). The genomic organization of *SCN5A* was deduced by comparing the cDNA with the genomic sequence. A glutamine residue at codon 1077 was numbered as the first amino acid residue of exon 18 according to NM_198056.1. This numbering was different from another reference sequence, NM_000335.3, a shorter 2015 amino acid splice variant which uses another potential acceptor site located 3-bp downstream from the original acceptor site of exon 18 and lacks the glutamine at position 1077 (1077delGln). According to Makielski *et al.* (2003), both splice variants with 2015 (65% of all transcripts) and 2016 amino acids residues (35% of all transcripts) are constitutively expressed.

PCR primers were designed in intronic regions to amplify all 27 coding exons (exons 2–28) (Table 1). The promoter regions and exon 1 were excluded because their structures were not fully characterized when this study began. Each exon was amplified by Ex-Taq (0.625 units) (Takara Shuzo, Tokyo, Japan) using the appropriate set of primers (0.2 μM) and genomic DNA (100 ng). The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 61°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 7 min. The PCR products were purified using a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH) and were directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The primers used to amplify each exon were also used for sequencing, except for the exons shown in Table 1. After the excess dye was removed with a DyeEx96 kit (Qiagen, Hilden, Germany), the eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). The novel variations were confirmed by repeating the PCR on the genomic DNA and sequencing the subsequent PCR products.

Statistical Analysis

SNP frequencies in both of the groups were assessed for deviation from Hardy-Weinberg equilibrium using the χ^2 test. The observed allele frequencies were all in Hardy-Weinberg equilibrium except for c.4299+116G>A (data not shown). The reason for the significant deviation from Hardy-Weinberg equilibrium of c.4299+116G>A ($p = 0.002$ for total subjects, $p = 0.014$ for patients, and $p = 0.059$ for controls) is currently unknown. This SNP was omitted from the haplotype inference. The comparison of allele frequencies between the patients and healthy controls was performed using the χ^2 test or Fisher's exact test as appropriate, and the differences were considered to be significant when $p < 0.05$. Pairwise linkage disequilibrium (LD) between each SNP was calculated by r^2 statistics. These analyses were performed by the SNPAlyze software (DYNACOM CO., Ltd., Kanagawa, Japan). Haplotype frequencies and diplotype configurations were estimated by LDSUPPORT software (Kitamura *et al.* 2002) using an expectation-maximization (EM) algorithm. To examine the differences in the overall haplotype frequency profile between the patients and controls, the global permutation test was performed according to the methods of Zhao *et al.* (2000) using the software PM+EH+ version 1.2 (model free analysis and permutation tests for allelic associations, http://linkage.rockefeller.edu/soft/list.html). When the global permutation test showed significance between the two groups, the differences in individual haplotype frequencies were evaluated by χ^2 test or Fisher's exact test. In these tests, the frequency of one haplotype was compared with the combined frequencies of all the other haplotypes for the patients and controls.

Results

SCN5A Variations Found in a Japanese Population

All the *SCN5A* coding exons (exons 2-28) and their flanking introns were sequenced in 166 Japanese

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Table 1 Primers used for amplification and sequencing of *SCN5A*

Exon	Forward primer (5′ to 3′)	Position[a]	Reverse primer (5′ to 3′)	Position[a]
2	GCAAATGGTGTCCCTCCCTC	38600581	ATGAGCCACCCTAAATAGAGC	38600037
	GGTCTGCCCACCCTGCTCTCT[b]	38600538		
3	GGGCAAGGCAGTGAGTCTAC	38597728	CTGGAGGAGGGTCAGAGGTT	38597234
			CTTAGGACCAGCAGGGAATC[b]	38597296
4	CAGCCCCAGTGTGTCTCCTT	38589746	GGCAGGACAGGGAGAAACTT	38589256
5	GAGCAAAGTTCCATCCCCAA	38588256	TGTCTCTCCCCACCAGGATG	38587773
6	ACTAGGCAATTTGTCGGCTC	38581161	ATGTCCACTGCCAATAGCCCC	38580702
	AGGTAAGATGCCCAGGTTTGCC[b]	38581092		
7	CCACCTCTGGTTGCCTACACTG	38577198	CTGTCCTCTGTCTGGGTCTCTG	38576709
	CACCCCAGCTCAACTCAGGC[b]	38577172	GGGATCAGGCAGGGCTTGAA[b]	38576732
8	GGCACTGGCAGCAGGATGTCT	38575550	GGGGTCAGGGCATAAATAGAA	38575170
	GGATGTCTTCAGAGGAACAG[b]	38575537		
9	CAGCGTGGCACTAGGTTTGT	38574074	AGTTTCTTTGCTGCTGATCC	38573618
10	TGGGACATCTCTACCCTCCT	38573396	CACCTATAGGCACCTACAGTCAG	38573004
11	GCCACTCCTATCTTCCTTCCTG	38572237	AACACCCAAAACTACCCTGT	38571668
	AAGTCACTGAGAGTTGCCTG[b]	38572112	GCTCCCCTACTCTAAGGAAG[b]	38571701
12	GCCCTCAATGCTCTGAGAAG	38571307	ACACAGTAGGTGCTCAACAA	38570719
13	CAGCATCCAGTGTCCCATCAAG	38566314	CAGTGTGGGGATGTCTAAAG	38565914
14	TCTCCCAGAGCAAGTCATAA	38565179	TGATTCCCACCCTCAAAAGA	38564714
15	CCACAGCCAAGCAAACCCCTA	38554839	GCCTTTCCTGCCTCTGTACC	38554327
16	GGGGGGAATAGGTGTCAGTG	38553289	GGGGGTAGGTGAAATAAATGAG	38552635
	TAGGTGTCAGTGCCCTCCAA[b]	38553281	CCAACTTACCACAAGGTTGC[b]	38552798
17	CCCTGGATTCAAGCCTCGGA	38548626	CTGTATATGTAGGTGCCTTATACATG	38548004
	CCTCAGTTTCCCCATCATAGAA[b]	38548571		
18	GGAGGAGTCTTCAGTGAGAT	38546762	TGACAGTGGCTGTGGCTCCCAA	38546340
			TGTGGCTCCCAACAGCAAAT[b]	38546350
19	TGACAGGCAAAAGTGGCTCT	38544061	ATCTAAGGCAGGGTGTTGGT	38543665
20	CTGCTCACCATATTGCCCTGTTC	38542709	GGGGGTCTGGAGAGCACATT	38542275
	GCCACCCCCATCATCGTAGCTC[b]	38542686		
21	GTGGCGGCAGGCATCTATAA	38533938	GCCTGGGTCACTCAGACTTACG	38533357
	AAATGGAAAGAAACGGTGCCTG[b]	38533782	ACTCAGACTTACGTCCTCCTTC[b]	38533366
22	CCCAGAAGCCAGGATACTCTTG	38529886	CCATGCTCCTACCAAGTCAGCC	38529388
23	CAGGGAGTTCATTCTTTCTTG	38527641	CCCTCTTCCTGCCCACATCAT	38527178
			CTGCCCACATCATGGGTGAT[b]	38527186
24	GTGAGGTGGGGTGGCTTGCTTT	38524528	AGGCTTGGGCATTCCAGAGA	38524191
25	ACACCCTCTTTCCCACAGAATG	38523817	GCAGGAGCAAGAAGAGGACCA	38523428
	ACAGAATGGACACCCCTAGAC[b]	38523803	CCAACAGGGAAGGTGAGATG[b]	38523452
26	GTGGTCAATCCTGGCATCCTCA	38522978	TTCCTCCCTATCTCTACGAG	38522595
27	TTTGGGCTCACTAGAGGGTAGA	38521785	CCCATTCCCAGACTCATCCTTG	38521076
28-1[c]	GCTCCTTGCCATATAGAGACC	38518782	GTGCTCTCCTCCGTGGCCACGC	38518140
	TGCACAGTGATGCTGGCTGGAA[b]	38518741	CAGTGTTGAGGATGGGGCTGAG[b]	38518318
28-2[c]	AAGTGGGAGGCTGGCATCGAC	38518441	CCGCCTGCTGACGGAAGAGGA	38517691
	TGGGAGGCTGGCATCGACGAC[b]	38518438		
28-3[c]	CCAACCAGATAAGCCTCATCAACA	38517999	AGCCCATTCACAACATATACAGTCT	38517122
	CATCCAAGATCTCCTACGAGCC[b]	38517837	CAGGCTGGTTTGTGACTGACTG[b]	38517351

[a]The nucleotide position of the 5′ end of each primer on NT_022517.16.
[b]The primers used for sequencing were indicated only when they were different from those used to amplify each exon.
[c]Three sets of overlapping primers were used to amplify exon 28.

arrhythmic patients and 232 healthy controls. We found 69 genetic variations, including 54 novel ones. The positions and frequencies of all variations found in both the patients and healthy controls are shown in Table 2. Of the 69 variations found in this study, 66 were single nucleotide polymorphisms (SNPs) and the remaining three were a deletion in intron 6 (c.611+76delC), an adenine base duplication in the polyadenine (poly A)

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Table 2 Summary of variations in the *SCN5A* gene detected in Japanese arrhythmic patients and healthy controls

Location	Position[a]	Nucleotide alterations	Amino acid alterations[b]	Allele Frequency (patient)	Allele Frequency (healthy control)	Statistics cases/control[c]	References[d]
Intron 1	−64	T>C		0/332 (0)	1/464 (0.002)	-	*
Exon 2	c.30	C>T	p.Thr10Thr	0/332 (0)	1/464 (0.002)	-	*
	c.87	G>A	p.Ala29Ala	126/332 (0.380)	160/464 (0.345)	N.S.	NCBI
Intron 3	c.393−113	T>C		0/332 (0)	1/464 (0.002)	-	*
Exon 4	c.453	C>T	p.His151His	1/332 (0.003)	1/464 (0.002)	-	*
Intron 4	c.482+165	C>T		1/332 (0.003)	0/464 (0)	-	*
	c.482+184	A>G		164/332 (0.494)	211/464 (0.455)	N.S.	NCBI
	c.483−33	C>T		0/332 (0)	1/464 (0.002)	-	*
Exon 5	c.552	C>T	p.His184His	1/332 (0.003)	0/464 (0)	-	*
Intron 5	c.611+74	C>G		0/332 (0)	1/464 (0.002)	-	*
	c.611+76	delC		0/332 (0)	1/464 (0.002)	-	*
Intron 6	c.703+130	G>A		46/332 (0.139)	40/464 (0.086)	$p = 0.019$	*
Exon 7	c.714	C>T	p.Thr238Thr	1/332 (0.003)	0/464 (0)	-	*
	c.801	C>T	p.Ile267Ile	1/332 (0.003)	0/464 (0)	-	*
Intron 7	c.934+5	G>A		0/332 (0)	1/464 (0.002)	-	*
Intron 8	c.998+33	T>C		0/332 (0)	1/464 (0.002)	-	*
	c.999-28	G>A		0/332 (0)	1/464 (0.002)	-	*
Intron 9	c.1140+98	A>G		12/332 (0.036)	19/464 (0.041)	N.S.	NCBI
	c.1141−3	C>A		22/332 (0.066)	45/464 (0.097)	N.S	1
Exon 10	c.1282	G>A	**p.Glu428Lys**	0/332 (0)	1/464 (0.002)	-	*
Intron 10	c.1339−24	G>A		23/332 (0.069)	48/464(0.103)	N.S.	NCBI
Intron 11	c.1518+39	C>T		1/332 (0.003)	0/464 (0)	-	*
	c.1519−68	C>T		8/332 (0.024)	12/464 (0.026)	N.S.	*
Exon 12	c.1595	T>G	**p.Phe532Cys**	1/332 (0.003)	0/464 (0)	-	*
	c.1673	A>G	**p.His558Arg**	24/332 (0.072)	48/464 (0.103)	N.S.	NCBI
	c.1755	C>T	p.His585His	1/332 (0.003)	0/464 (0)	-	*
Intron 13	c.2023+32	C>T		1/332 (0.003)	0/464 (0)	-	*
Exon 14	c.2066	G>A	**p.Arg689His**	1/332 (0.003)	0/464 (0)	-	*
	c.2102	C>T	**p.Pro701Leu**	1/332 (0.003)	0/464 (0)	-	*
	c.2151	G>A	p.Pro717Pro	1/332 (0.003)	0/464 (0)	-	*
Intron 15	c.2263+39	G>A		1/332 (0.003)	0/464 (0)	-	*
Intron 17	c.3229-61	C>T		0/332 (0)	1/464 (0.002)	-	*
Exon 18	c.3269	C>T	**p.Pro1090Leu**	12/332 (0.036)	11/464 (0.024)	N.S.	NCBI
Intron 18	c.3391−70	C>T		1/332 (0.003)	0/464 (0)	-	*
Exon 19	c.3442	G>A	**p.Ala1148Thr**	0/332 (0)	1/464 (0.002)	-	*
Exon 20	c.3556	G>A	**p.Ala1186Thr**	0/332 (0)	1/464 (0.002)	-	*
	c.3578	G>A	**p.Arg1193Gln**	21/332 (0.063)	29/464 (0.063)	N.S.	2
	c.3598	C>T	**p.His1200Tyr**	1/332 (0.003)	0/464 (0)	-	*
Intron 20	c.3667−89	dupA		24/332 (0.072)	29/464 (0.063)	N.S.	*
Intron 21	c.3840+17	G>A		1/332 (0.003)	1/464 (0.002)	-	*
	c.3840+73	G>A		24/332 (0.072)	29/464 (0.063)	N.S.	*
	c.3840+76	C>T		1/332 (0.003)	0/464 (0)	-	*
Intron 23	c.4246-7	C>A		0/332 (0)	1/464 (0.002)	-	*
Intron 24	c.4299+53	T>C		88/332 (0.265)	128/464 (0.276)	N.S.	3
	c.4299+116	G>A		36/332 (0.108)	47/464 (0.101)	N.S	*
Exon 25	c.4302	T>C	p.Tyr1434Tyr	0/332 (0)	3/464 (0.006)	-	*
Intron 25	c.4437+45	C>T		0/332 (0)	1/464 (0.002)	-	*
Intron 26	c.4542+86	A>G		38/332 (0.114)	52/464 (0.112)	N.S	*
	c.4543−30	T>G		0/332 (0)	1/464 (0.002)	-	*
Intron 27	c.4813 + 24	G>A		3/332 (0.009)	2/464 (0.004)	-	*
	c.4813+164	C>G		38/332 (0.114)	52/464 (0.112)	N.S.	NCBI
	c.4813+215	T>C		38/332 (0.114)	52/464 (0.112)	N.S.	NCBI
	c.4813+262	A>C		38/332 (0.114)	52/464 (0.112)	N.S.	NCBI

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Table 2 Continued

Location	Position[a]	Nucleotide alterations	Amino acid alterations[b]	Allele Frequency (patient)	Allele Frequency (healthy control)	Statistics cases/control[c]	References[d]
	c.4814−80	C>A		0/332 (0)	6/464 (0.013)	-	*
Exon 28	c.4851	C>T	p.Phe1617Phe	0/332 (0)	1/464 (0.002)	-	*
	c.4999	G>A	**p.Val1667Ile**	1/332 (0.003)	0/464 (0)	-	*
	c.5082	C>T	p.Phe1694Phe	0/332 (0)	1/464 (0.002)	-	*
	c.5216	G>A	**p.Arg1739Gln**	1/332 (0.003)	0/464 (0)	-	*
	c.5457	T>C	p.Asp1819Asp	164/332 (0.494)	230/464 (0.496)	N.S.	NCBI
	c.5737	C>T	**p.Arg1913Cys**	0/332 (0)	1/464 (0.002)	-	*
	c.5775	C>G	p.Ser1925Ser	1/332 (0.003)	2/464 (0.004)	-	*
	c.5795	C>T	**p.Ala1932Val**	0/332 (0)	1/464 (0.002)	-	*
	c.5851	G>T	**p.Val1951Leu**	1/332 (0.003)	2/464 (0.004)	-	3,4
	c.5963	T>G	**p.Leu1988Arg**	1/332 (0.003)	11/464 (0.024)	$p = 0.018$	*
3′-UTR	c.6056	C>T		0/332 (0)	1/464 (0.002)	-	*
	c.6122_6125	dupGTCA		2/332 (0.006)	1/464 (0.002)	-	*
	c.6155	G>C		1/332 (0.003)	0/464 (0)	-	*
	c.6174	A>G		163/332 (0.491)	229/464 (0.494)	N.S	NCBI
	c.6255	T>C		1/332 (0.003)	9/464 (0.019)	N.S.	*

[a]cDNA numbers are relative to the ATG start site and based on the cDNA sequence (NM_198056.1).
[b]Non-synonymous changes are shown in **bold**.
[c]p value of χ^2 test or Fisher's exact test for allele was shown. N.S.: not significant ($p \geqq 0.05$).
[d]* indicates a novel variation. "NCBI" denotes the SNPs that have already published in the dbSNP database of the National Center for Biotechnology Information. NCBI SNP cluster ID (rs#) of these SNPs are as follows; c.87G>A, p.Ala29Ala (rs6599230); c.482+184A>G (rs6781731); c.1140+98A>G (rs6599222); c.1339–24G>A (rs7428779); c.1673A>G, p.His558Arg (rs1805124); c.3269C>T, p.Pro1090Leu (rs1805125); c.4813+164C>G (rs7429347); c.4813+215T>C (rs7431641); c.4813+262A>C (rs7432127); c.5457T>C, p.Asp1819Asp (rs1805126); c.6174A>G (rs7429945).
The other SNPs that were reported elsewhere are as follows:
1, Schulze-Bahr *et al.* [2003], 2, Vatta *et al.* [2002], 3, Iwasa *et al.* [2000], 4, Priori *et al.* [2002].

tract of intron 20 (c.3667-89dupA), and a 4-nucleotide duplication in the 3′-untranslated region (3′-UTR, c.6122−6125dupGTCA). Of the 66 SNPs, 29 were located in the coding exons (13 synonymous and 16 nonsynonymous), 33 in the introns, and 4 in the 3′-UTR.

The 54 novel variations included twelve nonsynonymous SNPs. One SNP, c.5963T>G (p.Leu1988 Arg), was heterozygous in eleven healthy subjects and only one patient. Five SNPs, c.1282G>A (p.Glu428Lys), c.3442G>A (p.Ala1148Thr), c.3556 G>A (p.Ala1186Thr), c.5737C>T (p.Arg1913Cys), and c.5795C>T (p.Ala1932Val), were heterozygous in 5 different healthy controls. The remaining six nonsynonymous SNPs, c.1595T>G (p.Phe532Cys), c.2066 G>A (p.Arg689His), c.2102C>T (p.Pro701Leu), c.3598C>T (p.His1200Tyr), c.4999G>A (p.Val 1667Ile), and c.5216G>A (p.Arg1739Gln), were found separately in six arrhythmic patients, but not in healthy controls. The locations corresponding to the 16 nonsynonymous SNPs in the SCN5A protein are depicted in Figure 1. Table 3 summarizes the clinical characteristics of the individuals with the novel nonsynonymous SNPs.

Fifteen previously reported variations were detected in our study. They included four coding SNPs (cSNP), c.87G>A (p.Ala29Ala), c.1673A>G (p.His558Arg), c.3269C>T (p.Pro1090Leu), and c.5457T>C (p.Asp1819Asp), and seven non-coding SNPs (c.482+184A>G, c.1140+98A>G, c.1339–24G>A, c.4813+164C>G, c.4813+215T>C, c.4813+262A>C, and c.6174A>G), which were published in the dbSNP database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/SNP/index.html). Two SNPs, c.4299+53T>C and c.5851G>T (p.Val1951Leu), were previously identified in Japanese individuals by Iwasa *et al.* (2000). The remaining 2 SNPs were c.1141-3C>A (Schulze-Bahr *et al.* 2003) and

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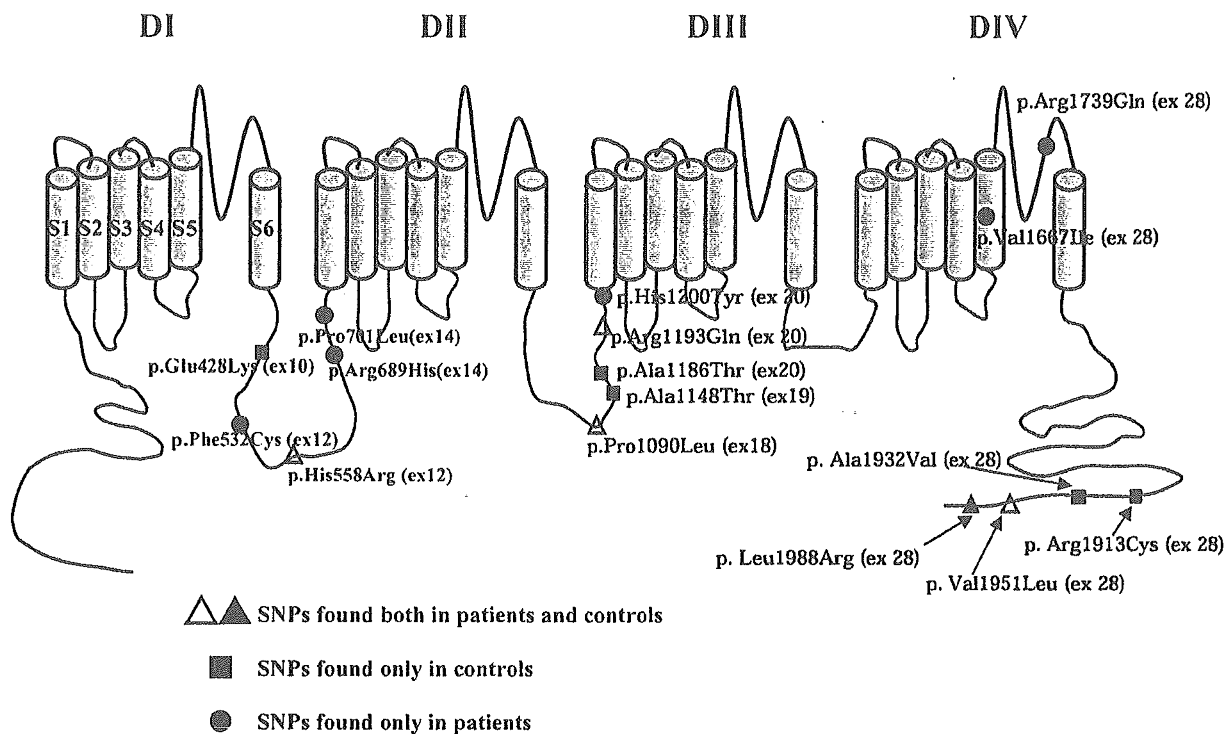

Figure 1 Sixteen nonsynonymous SNPs identified in the Japanese population in this study are depicted on the predicted topology of the *SCN5A* protein. One novel and four known nonsynonymous SNPs, found both in controls and patients, are indicated by an open triangle and closed triangle, respectively. Five novel nonsynonymous SNPs found only in controls are indicated by a closed square. Six novel nonsynonymous SNPs found only in patients are shown by a closed circle.

c.3578G>A (p.Arg1193Gln) (Vatta *et al.* 2002). The allele frequencies of c.5457C>T (p.Asp1819Asp) (0.50) and c.4299+53T>C (0.27) were similar to those (0.46 and 0.27, respectively) found in a Japanese population and reported by Iwasa *et al.* (2000). The frequencies of c.87G>A (p.Ala29Ala) (0.35) and c.1141-3C>A (0.10) for healthy controls were comparable to those (0.28 and 0.16, respectively) for 32 healthy Caucasians as reported by Paulussen *et al.* (2004).

On the other hand, we failed to detect the seven SNPs reported in the dbSNP database; c.100C>T (p.Arg34Cys; rs6791924), c.274–25G>A (rs7636280), c.274–24C>T (rs7627488), c.1654G>C (p.Gly552Arg; rs3918389), c.2437–97C>T (rs7645173), c.3183A>G (p.Glu1061Glu; rs7430407), and c.3305C>A (p.Ser1102Tyr; rs 7626962). Splawski *et al.* (2002) reported that p.Ser1102Tyr is a common variation of *SCN5A* in Africans at allele frequencies of 0.13–0.19, but is not found in Caucasians and Asians. p.Arg34Cys was found in a U.S. population at an allele frequency of 0.04 (Yang *et al.* 2002). Lastly, Paulussen *et al.* (2004) reported that the allele frequencies of c.274–24C>T and p.Glu1061Glu were 0.02 and 0.13, respectively, in 32 healthy Caucasians. Thus, they seem to be either ethnic-specific or rare.

Of the 69 variations, 22 were polymorphisms and detected with minor allele frequencies over 1% (more than 8 chromosomes from a total of 398 subjects). To determine whether these relatively frequent polymorphisms were associated with cardiac arrhythmias, their allele frequencies were compared between the patients and controls. Two out of the 22 SNPs showed significantly different frequencies between the patient and control groups ($p < 0.05$). The patients were more likely to have the c.703+130A allele compared with the healthy controls, with an odds ratio of 1.70 (95% confidence interval [95% CI] 1.07–2.65) and χ^2 of

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Table 3 Clinical characteristics of the arrhythmic patients and healthy individuals bearing novel nonsynonymous SNPs

Novel nonsynonymous SNPs	Age (years)	Sex	Diagnosis[a]	Medication[b]	Other nonsynonymous SNPs detected in the same subject[c]
c.1282G>A (p.Glu428Lys)	21	F	control subject	No	ND
c.1595T>G (p.Phe532Cys)	58	M	Paf, AT, MS	AMD 200 mg/day	ND
c.2066G>A (p.Arg689His)	54	M	VT, mitral valvular disease	MEX 300mg/day	ND
c.2102C>T (p.Pro701Leu)	60	M	Af	PIL 150 mg/day	c.5851G>T; p.Val1951Leu (hetero)
c.3442G>A (p.Ala1148Thr)	47	M	control subject	No	ND
c.3556G>A (p.Ala1186Thr)	42	M	control subject	No	ND
c.3598C>T (p.His1200Tyr)	36	M	VF, HCM	AMD 100 ng/day	ND
c.4999G>A (p.Val1667Ile)	66	F	VT	AMD 200mg/day	c.3269C>T; p.Pro1090Leu (hetero)
c.5216G>A (p.Arg1739Gln)	35	M	DCM, CHF	AMD 100 mg/day	c.1673A>G; p.His558Arg (hetero)
c.5737C>T (p.Arg1913Cys)	48	M	control subject	No	c.3578G>A; p.Arg1193Gln (hetero)
c.5795C>T (p.Ala1932Val)	35	M	control subject	No	ND
c.5963T>G (p.Leu1988Arg)	47	M	DCM, nonsustained VT, CHF, myocardial sarcoidosis	AMD 150 mg/day	c.1673A>G; p.His558Arg (homo)
	37	F	control subject	No	c.1673A>G; p.His558Arg (hetero)
	58	M	control subject	No	c.1673A>G; p.His558Arg (hetero)
	34	M	control subject	No	c.1673A>G; p.His558Arg (hetero)
	41	M	control subject	No	c.1673A>G; p.His558Arg (hetero)
	45	M	control subject	No	c.1673A>G; p.His558Arg (hetero)
	47	M	control subject	No	c.1673A>G; p.His558Arg (hetero)
	31	F	control subject	No	c.1673A>G; p.His558Arg (homo)
	35	F	control subject	No	c.1673A>G; p.His558Arg (hetero)
	30	F	control subject	No	c.1673A>G; p.His558Arg (hetero)
	21	M	control subject	No	c.1673A>G; p.His558Arg (hetero)
	28	M	control subject	No	c.1673A>G; p.His558Arg (hetero), 3578G>A; Arg1193Gln (hetero)

[a]Paf, paroxysmal atrial fibrillation; AT, atrial tachycardia; MS, mitral stenosis; VT, ventricular tachycardia; Af, atrial fibrillation; VF, ventricular fibrillation; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; CHF, congestive heart failure
[b]MEX, mexiletine; AMD, amiodarone; PIL, pilsicainide
[c]ND; not detected

5.50 ($p = 0.019$). In contrast, the allele frequency of c.5963T>G (p.Leu1988Arg) was significantly lower in the patients than in the controls (odds ratio = 0.124 [95% CI] 0.00–0.53, $p = 0.018$ by Fisher's exact test). As for the other 20 variations, no significant differences were found between the allele frequencies of the patients and healthy controls.

In our study, the control subjects (mean age, 40 ± 12 years) were relatively young compared with the arrhythmic patients (mean age, 58 ± 12 years). To evaluate the influence of age differences on our association results, we divided both the patients and healthy controls into three subgroups by age (i.e., 20–39, 40–59 and 60–80 years). Both c.703+130G>A and p.Leu1988Arg were detected at almost equal frequencies among the three age subgroups within each group (data not shown), indicating that the allele frequencies in either group were not influenced by the age of the subjects.

Linkage Disequilibrium (LD) Analysis

Pairwise LD was calculated between the 22 polymorphisms (minor allele frequency>1.0%). The pairs that have r^2 values over 0.1 are shown in Figure 2. Strong LDs were found in four SNP groups: c.1141−3C>A, c.1339-24G>A, and c.1673A>G (p.His558Arg) ($r^2>0.92$); c.4542+86A>G, c.4813+164C>G, c.4813+215T>C, and c.4813+262A>C ($r^2 = 1.0$); c.5457T>C (p.Asp1819Asp) and c.6174A>G ($r^2 = 0.99$); c.5963T>G (p.Leu1988Arg) and c.6255T>C ($r^2 = 0.83$). Moderate LD was observed between c.4299+53T>C and c.5457T>C (p.Asp1819Asp) ($r^2 = 0.37$), between c.4299+53T>C and c.6174A>G ($r^2 = 0.37$), between c.3269C>T (p.Pro1090Leu) and c.3840+73G>A ($r^2 = 0.27$), and between c.1519−68C>T and c.3667−89dupA ($r^2 = 0.25$).

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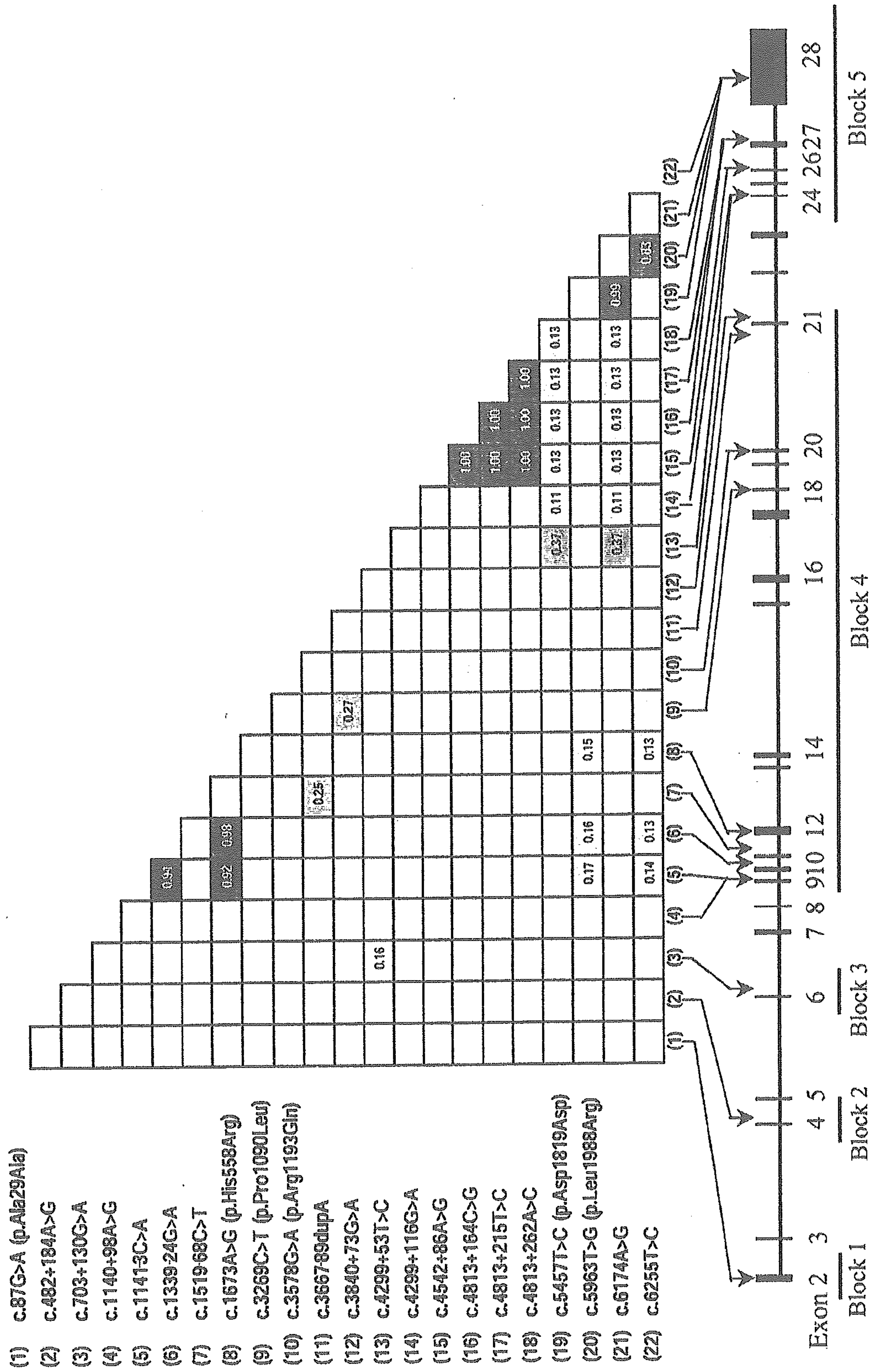

Figure 2 The pairwise LD between 22 variations found at allele frequencies over 1.0% for all 398 subjects. The LDs (r^2 values) are shown with a 10-graded blue colour, and the location of the variations is indicated in the schematic diagram of the *SCN5A* gene.

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Figure 1 also illustrates the positions of the 22 variations. For subsequent analysis of *SCN5A* haplotype structures we assigned 5 LD blocks, so that the closely associated SNPs ($\rho^2 > 0.20$) could be grouped together. Blocks 1, 2 and 3 included only a single SNP, c.87G>A (p.Ala29Ala), c.482+184A>G, and c.703+130G>A, respectively, because these SNPs showed little LD ($\rho^2 < 0.20$) with the other variations. Block 4, spanning 40 kb (from introns 9 to 21), included 9 variations (c.1140+98A>G, c.1141–3C>A, c.1339–24G>A, c.1519–68C>T, c.1673A>G; p.His558Arg, c.3269C>T; p.Pro1090Leu, c.3578G>A; p.Arg1193Gln, c.3667–89dupA, c.3840+73G>A). The remaining 10 variations (c.4299+53T>C, c.4299+116G>A, c.4542+86A>G, c.4813+164C>G, c.4813+215T>C, c.4813+262A>C, c.5457T>C; p.Asp1819Asp, c.5963T>G; p.Leu1988Arg, c.6174A>G, c.6255T>C) were assigned to block 5, ranging from intron 24 to exon 28 (7 kb).

Haplotypes and their Associations with Arrhythmogenesis

First, the haplotype frequencies for the blocks were evaluated. For blocks 1 to 3, the haplotype frequencies were the same as the allele frequencies of the single SNPs. Therefore, significant differences in haplotype (allele) frequencies were found only in block 3 (c.703+130G>A) as described above. In block 4, eight common haplotypes were inferred with frequencies over 1% (**1a*–**1e*, **2a*, **3a* and **4a*), accounting for 97% of all the observed haplotypes (Table 4a). In block 5, five common haplotypes with frequencies over 1% (**1a*–**1d*, and **2a*) accounted for 99% of all the inferred haplotypes (Table 4b). The frequency of the block 5 *2 haplotype (**2a* and **2b*) bearing c.5963T>G (p.Leu1988Arg) was about eight times higher in the controls than in the patients. For the other haplotypes in blocks 4 and 5, however, no significant differences in haplotype frequencies between the patients and controls were obtained.

Next, the combinations of in-block haplotypes (inter-block haplotypes; e.g., block1 **1a* - block2 **1a* – block3 **1a* – block4 **1a* – block5 **1a*) were assessed. However, there were too many inter-block haplotypes, each having low frequencies, to obtain statistical significance by comparing them between the patients and controls. Nonetheless, the haplotypes harbouring the SNPs c.703+130G>A or c.5963T>G (p.Leu1988Arg), which showed significant differences in allele frequencies between the patients and controls, showed unique linkages beyond the blocks. The *2 haplotypes (**2a* and **2b*) in block 5 were always associated with the **2a* haplotype in block 4, which harbours 3 linked SNPs, including the nonsynonymous SNP c.1673A>G (p.His558Arg). The SNP c.703+130G>A in block 3 was mostly associated with **1b* in block 5, which harboured three SNPs including c.4299+53T>C. Thus, in spite of recombination between LD blocks, some block haplotype combinations were sustained. To assess the association between these inter-block haplotypes and risk of arrhythmias, we then applied the permutation and model-free analysis and estimation haplotype (PM+EM+) methods using the two haplotype-tagging SNPs c.1673A>G (p.His558Arg) and c.5963T>G (p.Leu1988Arg) or c.703+130G>A and c.4299+53T>C. (Table 5).

When the combinations between c.1673A>G (p.His558Arg) and c.5963T>G (p.Leu1988Arg) were analyzed, 3 haplotypic combinations were inferred in both of the two groups. The haplotype AG (558His-1988Arg) was completely absent in both groups, and the haplotype GG (558Arg-1988Arg) was present less frequently in the patients compared to the healthy controls. The global permutation test indicated that there was a significant difference in distribution of the haplotypes between the patients and controls ($\chi^2 = 7.42$, $p = 0.0260$). In accordance with this result, the frequency of haplotype GG (558Arg-1988Arg) was significantly lower in the patients than in the controls ($p = 0.018$).

As for the 4 haplotypes estimated from c.703+130G>A and c.4299+53T>C, haplotype AT was about 3 times as frequent in the patients as in the controls. However, the global difference between the patients and controls had a borderline significance ($\chi^2 = 8.64$, $p = 0.0550$) by the global permutation test.

Discussion

Since the defect in *SCN5A* was first reported to be a cause of LQT-3 (Wang *et al.* 1995), a variety of genetic alterations in *SCN5A* have been suggested to influence the pathophysiology of cardiac arrhythmias and/or pharmacological sensitivities to antiarrhythmic drugs. In this study we comprehensively searched for

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Table 4 Haplotypes of blocks 4 and 5 and their frequencies in *SCN5A* for Japanese arrhythmic patients and healthy controls

a) block 4

Exon/Intron	Intron 9	Intron 9	Intron 10	Intron 11	Exon 12	Exon 18	Exon 20	Intron 20	Intron 21	Frequency (%) All subjects	Patients	Controls
Position (NT_022517.16)	g.38573687	g.38573267	g.38572048	g.38571267	g.38571045	g.38546571	g.38542501	g.38533787	g.38533452			
Position (NM_198056.1)	c.1140 + 98	c.1141 − 3	c.1339 − 24	c.1519 − 68	c.1673	c.3269	3578	c.3667 − 89	c.3840 + 73			
Nucleotide change	A>G	C>A	G>A	C>T	A>G	C>T	G>A	dupA	G>A			
Amino acid change					His558Arg	Pro1090Leu	Arg1193Gln					
Haplotypes[a]												
*1 *1a										68.22	69.57	68.50
*1b									■	4.11	3.88	3.92
*1c								■		3.80	4.45	2.94
*1d	■									3.73	3.54	3.40
*1e				■				■		1.94	1.78	1.82
*1f				■						0.29	0.32	0.26
*2 *2a		■	■		■					7.41	5.36	8.58
*2b		■	■		■			■		0.47	0.26	0.71
*2c			■		■					0.40	0.30	0.45
*3 *3a							■			6.06	6.04	5.53
*4 *4a						■			■	2.09	2.59	1.56
*4b						■				0.29	0.32	0.25
*5 *5a		■	■		■	■			■	0.28	0.70	0.02
Others with frequencies less than 0.25 %										0.91	0.89	2.06

b) block 5

Exon/Intron	Intron 24	Intron 26	Intron 27	Intron 27	Intron 27	Exon 28	Exon 28	Exon 28 (3′-UTR)	Exon 28 (3′-UTR)	Frequency (%) All subjects	Patients	Controls
Position (NT_022517.16)	g.38524294	g.38522686	g.38521231	g.38521180	g.38521133	g.38518031	g.38517525	g.38517314	g.38517233			
Position (NM_198056.1)	c.4299 + 53	c.4542 + 86	c.4813 + 164	c.4813 + 215	c.4813 + 262	5457	5963	c.6174	c.6255			
Nucleotide change	T>C	A>G	C>G	T>C	A>C	T>C	T>G	A>G	T>C			
Amino acid change						Asp1819Asp (silent)	Leu1988Arg					
Haplotypes[a]												
*1 *1a										48.85	50.30	47.82
*1b	■						■	■		26.99	26.51	27.35
*1c		■	■	■	■	■		■		11.31	11.45	11.21
*1d						■		■		10.95	11.14	10.80
*1e						■				0.25	0.30	0.22
*2 *2a							■		■	1.26	0.30	1.94
*2b							■			0.25	0.00	0.43
Others with frequencies less than 0.25 %)										0.14	0.00	0.23

[a]The wild-type haplotype was designated as *1, and the nonsynonymous SNP-bearing haplotypes were numerically defined. The subtypes were named with small alphabetical letters in the order of their frequencies.
White cells indicate major allele, and ■ indicate minor allele.
The haplotypes found in two or more chromosomes (frequencies>0.25%) were shown.

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Table 5 Haplotype frequencies of two haplotype-tagging SNPs and their associations with the risk of arrhythmias

c.1673A>G (p.His558Arg) and c.5963T>G (p.Leu1988Arg)

	Selected locus		Haplotype frequency (%)			
Haplotype	c.1673A>G (p.His558Arg)	c.5963T>G (p.Leu1988Arg)	All subjects	Patients	Controls	Statistics
AT	A (His558)	T (Leu1988)	90.95	92.77	89.66	N.S.
GT	G (Arg558)	T (Leu1988)	7.54	6.93	7.97	N.S.
AG	A (His558)	G (Arg1988)	0.00	0.00	0.00	-
GG	G (Arg558)	G (Arg1988)	1.51	0.30	2.37	$p = 0.0181$
			Global permutation test[a]	$\chi^2 = 7.42, p = 0.0260$		

c.703+130G>A and c.4299 + 53T>C

	Selected locus		Haplotype frequency (%)		
Haplotype	c.703+130G>A	c.4299+53T>C	All subjects	Patients	Controls
GT	G	T	70.55	69.89	71.06
AT	A	T	2.32	3.60	1.35
GC	G	C	18.65	16.25	20.31
AC	A	C	8.49	10.25	7.27
			Global permutation test[a]	$\chi^2 = 8.64, p = 0.0550$	

[a]The empirical p values were obtained after 1000 permutations.

genetic variations in *SCN5A*. A total of 69 variations were detected in 166 unrelated Japanese arrhythmic patients and 232 healthy controls. Among them, 54 variations were novel and 15 were previously identified.

The fifteen reported variations include 4 nonsynonymous SNPs (p.His558Arg, p.Pro1090Leu, p.Arg1193Gln, and p.Val1951Leu). p.His558Arg has been extensively studied in various ethnic groups. Yang *et al.* (2002) have compared the frequency of p.His558Arg in patients diagnosed with the drug-associated LQT syndrome with those in three control populations: patients tolerating QT-prolonging drugs, and populations in Tennessee and accross the United States. p.His558Arg was detected at similar allele frequencies of 0.18–0.24 in all populations. As for the Japanese population, its frequencies were reported to be 0.132 for 56 healthy controls and 0.08 for 50 individuals by Takahata *et al.* (2003) and Iwasa *et al.* (2000), respectively. In our study, the allele frequency of p.His558Arg in arrhythmic patients (0.072) was slightly lower than that in the healthy controls (0.103), although a significant difference was not obtained ($p = 0.1307$). p.Pro1090Leu is a well-known nonsynonymous SNP localized in the linker between DII and DIII. The frequency of p.Pro1090Leu was 12/334 (0.036) and 11/464 (0.024) for the patients and the controls, respectively, and comparable to that reported in the Japanese (0.04) by Iwasa *et al.* (2000). Vatta *et al.* (2002) reported that p.Arg1193Gln accelerated the fast inactivation of the Na^{+} channel *in vitro*. In our study, p.Arg1193Gln showed similar allele frequencies of 0.063 both in patients and healthy controls. From these results, it is likely that these three common SNPs are not directly involved in arrhythmogenesis in the Japanese. p.Val1951Leu, found at low frequencies in both patients (0.003) and healthy controls (0.004), was also detected at a similar frequency (0.005) by Iwasa *et al.* (2000). Although this SNP was reported in patients with BrS by Priori *et al.* (2002) and Mok *et al.* (2004), p.Val1951Leu alone is unlikely to be directly related with the pathogenesis of BrS.

Six novel nonsynonymous SNPs (p.Phe532Cys, p.Arg689His, p.Pro701Leu, p.His1200Tyr, p.Val1667Ile, and p.Arg1739Gln) were found separately in six arrhythmic patients. When flanking amino acid sequences of these SNPs were aligned with related

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sodium channel sequences (Supplementary Figure 1, online), the 6 amino acid residues of SCN5A, 532Phe, 689Arg, 701Pro, 1200His, 1667Val, and 1739Arg, were found top be highly conserved among the different Na^+ channel α subunit isoforms. Amino acid 532Phe, 689Arg, and 701Pro are localized in the linker between DI and DII, where the mutations associated with inherited arrhythmia syndrome such as p.Gly514Cys, p.Leu567Gln and p.Leu619Phe have already been reported (Tan *et al.* 2003). 1200His is located in the linker between DII and DIII, where the BrS-related variation p.Arg1193Gln (Vatta *et al.* 2002) is localized. 1667Val in DIVS5 and 1739Arg in the P loop of DIV form a pore structure together with DIVS6, where sodium channel blocking drugs are thought to bind. p.Arg1739Gln is a mutation located next to p.Gly1740Arg that was identified in a patient with BrS by Priori *et al.* (2002). Since these 4 novel nonsynonymous SNPs were not detected in 232 healthy controls, it is possible that they are related to cardiac arrhythmia pathogenesis, although these patients were not diagnosed with LQT or BrS. Some patients who carry these novel nonsynonymous SNPs simultaneously have p.His558Arg, p.Pro1090Leu or p.Val1951Leu (Table 3). At present it is not clear whether 1739Gln and 558Arg, 701Leu and 1951Leu or 1667Ile and 1090Leu are on the same chromosome. If they are, the interactions between the substituted residues may affect the biological properties of the Na^+ channels.

As for the five other novel nonsynonymous SNPs detected in healthy controls p.Glu428Lys is localized in the linker between DI and DII, p.Ala1148Thr and p.Ala1186Thr in the linker between DII and DIII, and p.Arg1913Cys and p.Ala1932Val in the C-terminal intracellular loop. 428Glu, 1148Ala and 1913Arg are well conserved, while 1186Ala and 1932Ala are not conserved (Supplementary Figure 1, online). Because the healthy individuals who carry these SNPs failed to show symptoms of arrhythmias, these SNPs are unlikely to affect Na^+ channel function. However, it has been recently suggested that some variations in *SCN5A* may increase the risk of acquired long QT syndrome triggered by drug administration in healthy individuals, who are clinically asymptomatic and have normal QT intervals under normal circumstances. Therefore, the possibility cannot be excluded that these five novel nonsynonymous SNPs in healthy controls influence the susceptibility to cardiac ion channel blockade and QT prolongation. We have begun analyzing the electrophysiological properties of the novel variations using heterologous expression systems. These studies will elucidate the importance of these variable sites in Na^+ channel function.

Another novel nonsynonymous SNP, p.Leu1988Arg, was heterozygous in eleven healthy subjects and one patient, and the allele frequency of p.Leu1988Arg was significantly lower in the patients than in the controls by Fisher's extract test ($p = 0.018$). Since all twelve subjects with p.Leu1988Arg also have p.His558Arg (Table 3), weak LD ($r^2 = 0.15$) was shown between p.His558Arg and p.Leu1988Arg (Figure 2). Haplotype analysis using the 2 SNPs, c.1673A>G (p.His558Arg) and c.5963T>G (p.Leu1988Arg), also showed that the frequency of the haplotype GG (558Arg–1988Arg) was significantly lower in the patients than in the healthy controls. These results suggest that the haplotype GG has been positively selected because of its protective effect against arrhythmias.

Although physiological characterization of Na^+ channels containing both p.His558Arg and p.Leu1988Arg are necessary to elucidate the negative association of the haplotype GG (558Arg-1988Arg) with cardiac arrhythmias, some underlying mechanisms could be speculated. 1988Leu is localized in the C-terminal intracellular loop but is not conserved among different Na^+ channel isoforms (Supplementary Figure 1, online). However, Cormier *et al.* (2002) reported that truncation of the distal region of the C-terminus (1921Leu stop mutant) reduced peak currents without affecting channel gating, by whole cell patch clamp recordings. Thus, it cannot be excluded that 1988Leu may be involved in regulating the density of functional Na^+ channels in the surface membrane. On the other hand, it has been postulated that p.His558Arg modulates functional changes of the Na^+ channel caused by other variations, and plays a role in intragenic complementation, although this common variation itself does not alter the voltage dependence of activation and inactivation kinetics of wild-type channels. For instance, Ye *et al.* (2003) showed that p.His558Arg restored the trafficking defect caused by the LQT-3 variation, p.Met1766Leu. Viswanathan *et al.* (2003) reported that p.His558Arg could attenuate

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the abnormal gating effect caused by the proximal variation, p.Thr512Ile, *in vitro*. The favourable channel modulation by 1988Arg in conjunction with 558Arg for protection against arrhythmias might result in the positive selection of the GG (558Arg–1988Arg) haplotype in the controls. Alternatively, 558Arg could restore any functional deterioration caused by 1988Arg. If the intragenic complementation by 558Arg acts on 1988Arg, the rare frequency of the AG (558His–1988Arg) haplotype, and the preference of the GG (558Arg–1988Arg) haplotype, in the controls is reasonable. Another possible explanation is that other variations, which reside in the GG (558Arg-1988Arg) haplotype, such as c.6255T>C in the 3′-UTR, might change the stability of the mRNA leading to a protective effect on arrhythmias. In this regard Yang *et al.* (2004) demonstrated that –92C>A located in the promoter of *SCN5A* increased luciferase activity in neonatal cardiac myocytes. They proposed that this polymorphism might represent the first example of an allele that could protect against serious arrhythmias.

Comparison of the allele frequencies between patients and controls clearly suggests that c.703 + 130G>A is associated with an increased risk of arrhythmias, although the functional role of this SNP remains unknown. Because the haplotype AT in patients was 3 times as common as in the controls, we could not rule out the possibility that c.703+130G>A is linked with an unidentified variation that influences susceptibility to cardiac arrhythmias. In the dbSNP database of NCBI many SNPs were reported in introns 5 and 6, proximal to c.703+130G>A, including rs6797133, rs6786119, rs6776383, rs6791081 and rs6793943. Further analyses are needed to reveal a haplotype containing c.703+130G>A that increases the risk for arrhythmias.

In conclusion, 69 genetic variations, including 54 novel ones, were detected in *SCN5A*. Eleven novel missense variations were found in eleven different individuals, of which 6 were found in arrhythmic patients and 5 were in healthy controls. Another novel missense variation (p.Leu1988Arg) was found in the patients at a significantly lower frequency than in the healthy controls ($p<0.05$). Furthermore, the frequency of a novel intronic SNP, c.703+130G>A, was significantly higher in the patients than in the controls. The analysis of LD and haplotype structures of *SCN5A* revealed the possibility that the haplotype harbouring p.Leu1988Arg and p.His558Arg is associated with protection against arrhythmias. These results indicate that some genetic variations and haplotypes of *SCN5A* are positively or negatively associated with cardiac rhythm disturbance in Japanese. These findings provide fundamental information necessary to further elucidate the effects of genetic variations of *SCN5A* on channel function and cardiac rhythm.

Acknowledgments

This work was supported in part by the Program for Promotion of Fundamental Studies in Health Sciences (MPJ -3 and -6) of the Pharmaceuticals and Medical Devices Agency (PMDA).

References

Ackerman, M. J., Siu, B. L., Sturner, W. Q., Tester, D. J., Valdivia, C. R., Makielski, J. C. & Towbin, J. A. (2001) Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. *JAMA* **286**, 2264–2269.

Akai, J., Makita, N., Sakurada, H., Shirai, N., Ueda, K., Kitabatake, A., Nakazawa, K., Kimura, A. & Hiraoka, M. (2000) A novel SCN5A mutation associated with idiopathic ventricular fibrillation without typical ECG findings of Brugada syndrome. *FEBS Lett* **479**, 29–34.

Balser, J. R. (1999) Structure and function of the cardiac sodium channels. *Cardiovasc Res* **42**, 327–338.

Balser, J. R. (2001) Inherited sodium channelopathies: novel therapeutic and proarrhythmic molecular mechanisms. *Trends Cardiovasc Med* **11**, 229–237.

Bezzina, C. R., Rook, M. B. & Wilde, A. A. (2001) Cardiac sodium channel and inherited arrhythmia syndromes. *Cardiovasc Res* **49**, 257–271.

Chen, Q., Kirsch, G. E., Zhang, D., Brugada, R., Brugada, J., Brugada, P., Potenza, D., Moya, A., Borggrefe, M., Breithardt, G., Ortiz-Lopez, R., Wang, Z., Antzelevitch, C., O'Brien, R. E., Schulze-Bahr, E., Keating, M. T., Towbin, J. A. & Wang, Q. (1998) Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature* **392**, 293–296.

Cormier, J. W., Rivolta, I., Tateyama, M., Yang, A. S. & Kass, R. S. (2002) Secondary structure of the human cardiac Na + channel C terminus: evidence for a role of helical

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structures in modulation of channel inactivation. *J Biol Chem* **277**, 9233–9241.

Fujiki, A., Usui, M., Nagasawa, H., Mizumaki, K., Hayashi, H. & Inoue, H. (1999) ST segment elevation in the right precordial leads induced with class IC antiarrhythmic drugs: insight into the mechanism of Brugada syndrome. *J Cardiovasc Electrophysiol* **10**, 214–218.

Gellens, M. E., George, A. L., Jr., Chen, L. Q., Chahine, M., Horn, R., Barchi, R. L. & Kallen, R. G. (1992) Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. *Proc Natl Acad Sci U S A* **89**, 554–558.

Iwasa, H., Itoh, T., Nagai, R., Nakamura, Y. & Tanaka, T. (2000) Twenty single nucleotide polymorphisms (SNPs) and their allelic frequencies in four genes that are responsible for familial long QT syndrome in the Japanese population. *J Hum Genet* **45**, 182–183.

Kitamura, Y., Moriguchi, M., Kaneko, H., Morisaki, H., Morisaki, T., Toyama, K. & Kamatani, N. (2002) Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. *Ann Hum Genet* **66**, 183–193.

Makielski, J. C., Ye, B., Valdivia, C. R., Pagel, M. D., Pu, J., Tester, D. J. & Ackerman, M. J. (2003) A ubiquitous splice variant and a common polymorphism affect heterologous expression of recombinant human SCN5A heart sodium channels. *Circ Res* **93**, 821–828.

Makita, N., Horie, M., Nakamura, T., Ai, T., Sasaki, K., Yokoi, H., Sakurai, M., Sakuma, I., Otani, H., Sawa, H. & Kitabatake, A. (2002) Drug-induced long-QT syndrome associated with a subclinical *SCN5A* mutation. *Circulation* **106**, 1269–1274.

Mok, N. S., Priori, S. G., Napolitano, C., Chan, K. K., Bloise, R., Chan, H. W., Fung, W. H., Chan, Y. S., Chan, W. K., Lam, C., Chan, N. Y. & Tsang, H. H. (2004) Clinical profile and genetic basis of Brugada syndrome in the Chinese population. *Hong Kong Med J* **10**, 32–37.

Paulussen, A. D., Gilissen, R. A., Armstrong, M., Doevendans, P. A., Verhasselt, P., Smeets, H. J., Schulze-Bahr, E., Haverkamp, W., Breithardt, G., Cohen, N. & Aerssens, J. (2004) Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. *J Mol Med* **82**, 182–188.

Priori, S. G., Napolitano, C., Gasparini, M., Pappone, C., Della Bella, P., Giordano, U., Bloise, R., Giustetto, C., De Nardis, R., Grillo, M., Ronchetti, E., Faggiano, G. & Nastoli, J. (2002) Natural history of Brugada syndrome: insights for risk stratification and management. *Circulation* **105**, 1342–1347.

Schott, J. J., Alshinawi, C., Kyndt, F., Probst, V., Hoorntje, T. M., Hulsbeek, M., Wilde, A. A., Escande, D., Mannens, M. M. & Le Marec, H. (1999) Cardiac conduction defects associate with mutations in *SCN5A*. *Nat Genet* **23**, 20–21.

Schulze-Bahr, E., Eckardt, L., Breithardt, G., Seidl, K., Wichter, T., Wolpert, C., Borggrefe, M. & Haverkamp, W. (2003) Sodium channel gene (*SCN5A*) mutation in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease. *Hum Mutat* **21**, 651–652.

Schwartz, P. J., Priori, S. G., Locati, E. H., Napolitano, C., Cantu, F., Towbin, J. A., Keating, M. T., Hammoude, H., Brown, A. M. & Chen, L. S. (1995) Long QT syndrome patients with mutations of the *SCN5A* and HERG genes have differential responses to Na+channel blockade and to increases in heart rate. Implications for gene-specific therapy. *Circulation* **92**, 3381–3386.

Splawski, I., Timothy, K. W., Tateyama, M., Clancy, C. E., Malhotra, A., Beggs, A. H., Cappuccio, F. P., Sagnella, G. A., Kass, R. S. & Keating, M. T. (2002) Variant of *SCN5A* sodium channel implicated in risk of cardiac arrhythmia. *Science* **297**, 1333–1336.

Takahata, T., Yasui-Furukori, N., Sasaki, S., Igarashi, T., Okumura, K., Munakata, A. & Tateishi, T. (2003) Nucleotide changes in the translated region of *SCN5A* from Japanese patients with Brugada syndrome and control subjects. *Life Sci* **72**, 2391–2399.

Tan, H. L., Bezzina, C. R., Smits, J. P., Verkerk, A. O. & Wilde, A. A. (2003) Genetic control of sodium channel function. *Cardiovasc Res* **57**, 961–973.

Vatta, M., Dumaine, R., Varghese, G., Richard, T. A., Shimizu, W., Aihara, N., Nademanee, K., Brugada, R., Brugada, J., Veerakul, G., Li, H., Bowles, N. E., Brugada, P., Antzelevitch, C. & Towbin, J. A. (2002) Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. *Hum Mol Genet* **11**, 337–345.

Viswanathan, P. C., Benson, D. W. & Balser, J. R. (2003) A common *SCN5A* polymorphism modulates the biophysical effects of an *SCN5A* mutation. *J Clin Invest* **111**, 341–346.

Wang, Q., Shen, J., Splawski, I., Atkinson, D., Li, Z., Robinson, J. L., Moss, A. J., Towbin, J. A. & Keating, M. T. (1995) *SCN5A* mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* **80**, 805–811.

Wang, Q., Li, Z., Shen, J. & Keating, M. T. (1996) Genomic organization of the human *SCN5A* gene encoding the cardiac sodium channel. *Genomics* **34**, 9–16.

Yang, P., Kanki, H., Drolet, B., Yang, T., Wei, J., Viswanathan, P. C., Hohnloser, S. H., Shimizu, W., Schwartz, P. J., Stanton, M., Murray, K. T., Norris, K., George, A. L., Jr. & Roden, D. M. (2002) Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. *Circulation* **105**, 1943–1948.

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Yang, P., Kupershmidt, S. & Roden, D. M. (2004) Cloning and initial characterization of the human cardiac sodium channel (*SCN5A*) promoter. *Cardiovasc Res* **61**, 56–65.
Ye, B., Valdivia, C. R., Ackerman, M. J. & Makielski, J. C. (2003) A common human *SCN5A* polymorphism modifies expression of an arrhythmia causing mutation. *Physiol Genomics* **12**, 187–193.
Zhao, J. H., Curtis, D. & Sham, P. C. (2000) Model-free analysis and permutation tests for allelic associations. *Hum Hered* **50**, 133–139.

Received: 9 August 2004
Accepted: 5 November 2004

Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/ahg/ahg167/ahg167sm.htm

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Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 46, No. 7, 2005
ISSN 0735-1097/05/$30.00
doi:10.1016/j.jacc.2005.05.077

Malignant Entity of Idiopathic Ventricular Fibrillation and Polymorphic Ventricular Tachycardia Initiated by Premature Extrasystoles Originating From the Right Ventricular Outflow Tract

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OBJECTIVES The aim of this study was to assess the clinical characteristics and the efficacy of radiofrequency catheter ablation (RFCA) for idiopathic ventricular fibrillation (VF) and/or polymorphic ventricular tachycardia initiated by ventricular extrasystoles originating from the right ventricular outflow tract (RVOT).

BACKGROUND Ventricular fibrillation and/or polymorphic ventricular tachycardia are occasionally initiated by ventricular extrasystoles originating from the RVOT in patients without structural heart disease.

METHODS Among 101 patients without structural heart disease in whom RFCA was conducted for idiopathic ventricular tachyarrhythmias arising from the RVOT, we examined the clinical characteristics and the efficacy of RFCA in 16 patients with spontaneous VF and/or polymorphic ventricular tachycardia initiated by the ventricular extrasystoles originating from the RVOT.

RESULTS Among 16 patients, spontaneous episodes of VF were documented in 5 patients, and 11 patients had prior episodes of syncope. Holter recordings showed frequent isolated ventricular extrasystoles with the same morphology as that of initiating ventricular extrasystoles, and non-sustained polymorphic ventricular tachycardia with short cycle length (mean of 245 ± 28 ms) in all 16 patients. Radiofrequency catheter ablation by targeting the initiating ventricular extrasystoles eliminated episodes of syncope, VF, and cardiac arrest in all patients during follow-up periods of 54 ± 39 months.

CONCLUSIONS Our data suggest that the malignant entity of idiopathic VF and/or polymorphic ventricular tachycardia was occasionally present in patients with idiopathic ventricular arrhythmias arising from the RVOT. Radiofrequency catheter ablation was effective as a treatment option for this entity. (J Am Coll Cardiol 2005;46:1288–94) © 2005 by the American College of Cardiology Foundation

Ventricular fibrillation (VF) and polymorphic ventricular tachycardia (PVT) are malignant arrhythmias resulting in sudden cardiac death (1–5). Recent studies by Haissaguerre

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et al. (6,7) reported that idiopathic VF initiated by dominant triggers from distal Purkinje system or right ventricular outflow tract (RVOT) was successfully eliminated by radiofrequency catheter ablation (RFCA).

Although idiopathic ventricular tachycardia and ventricular extrasystoles (VE) originating from the RVOT in patients without structural heart diseases are considered benign (8–12), VF and/or PVT are occasionally initiated by VE originating from the RVOT.

The present study is designed to assess the clinical characteristics and the efficacy of RFCA for the malignant entity of idiopathic VF and/or PVT initiated by VE originating from the RVOT.

METHODS

Patient characteristics. Sixteen patients who showed spontaneous VF and/or PVT initiated by the VE with left bundle branch block morphology and inferior axis in their clinical course (VF/PVT group) were enrolled in this study among 101 consecutive patients in whom RFCA was conducted for treatment of ventricular tachyarrhythmias arising from the RVOT. There were seven men and nine women ranging in age from 25 to 54 years (mean of 39 ± 10 years). In all patients, physical examination, chest roentgenogram, laboratory values, treadmill exercise test, echocardiographic study with wall motion analysis, Doppler screening, and signal-averaged electrocardiogram (SAECG) were per-

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Manuscript received February 9, 2005; revised manuscript received April 28, 2005, accepted May 9, 2005.

Abbreviations and Acronyms

ECG	= electrocardiogram
EPS	= electrophysiologic study
ICD	= implantable cardioverter-defibrillator
PVC	= premature ventricular contraction
PVT	= polymorphic ventricular tachycardia
RFCA	= radiofrequency catheter ablation
RVOT	= right ventricular outflow tract
SAECG	= signal-averaged electrocardiogram
VE	= ventricular extrasystoles
VF	= ventricular fibrillation

formed, and no structural heart disease was found. Patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia (13) or Brugada syndrome (14) were excluded from this study. During hospitalization, the patients had frequent VE identical to the initiating beat of VF/PVT recorded by Holter recording or monitoring electrocardiogram (ECG) so that we could recognize the 12-lead QRS morphology of the initiating beats (Fig. 1A). In some cases, non-sustained PVT initiated by the VE of the RVOT origin could be recorded in the 12-lead ECG (Fig. 1B). We compared the clinical characteristics between the 16 patients with VF/PVT group and the remaining 85 patients in whom RFCAs were conducted for treatment of idiopathic monomorphic ventricular tachycardia arising from the RVOT (RVOT-VT group). Ventricular fibrillation was defined as a polymorphic ventricular tachyarrhythmia with hemodynamic decompensation requiring direct cardioversion for termination. Polymorphic ventricular tachycardia was defined as more than five consecutive beats with different QRS morphology and terminating spontaneously.

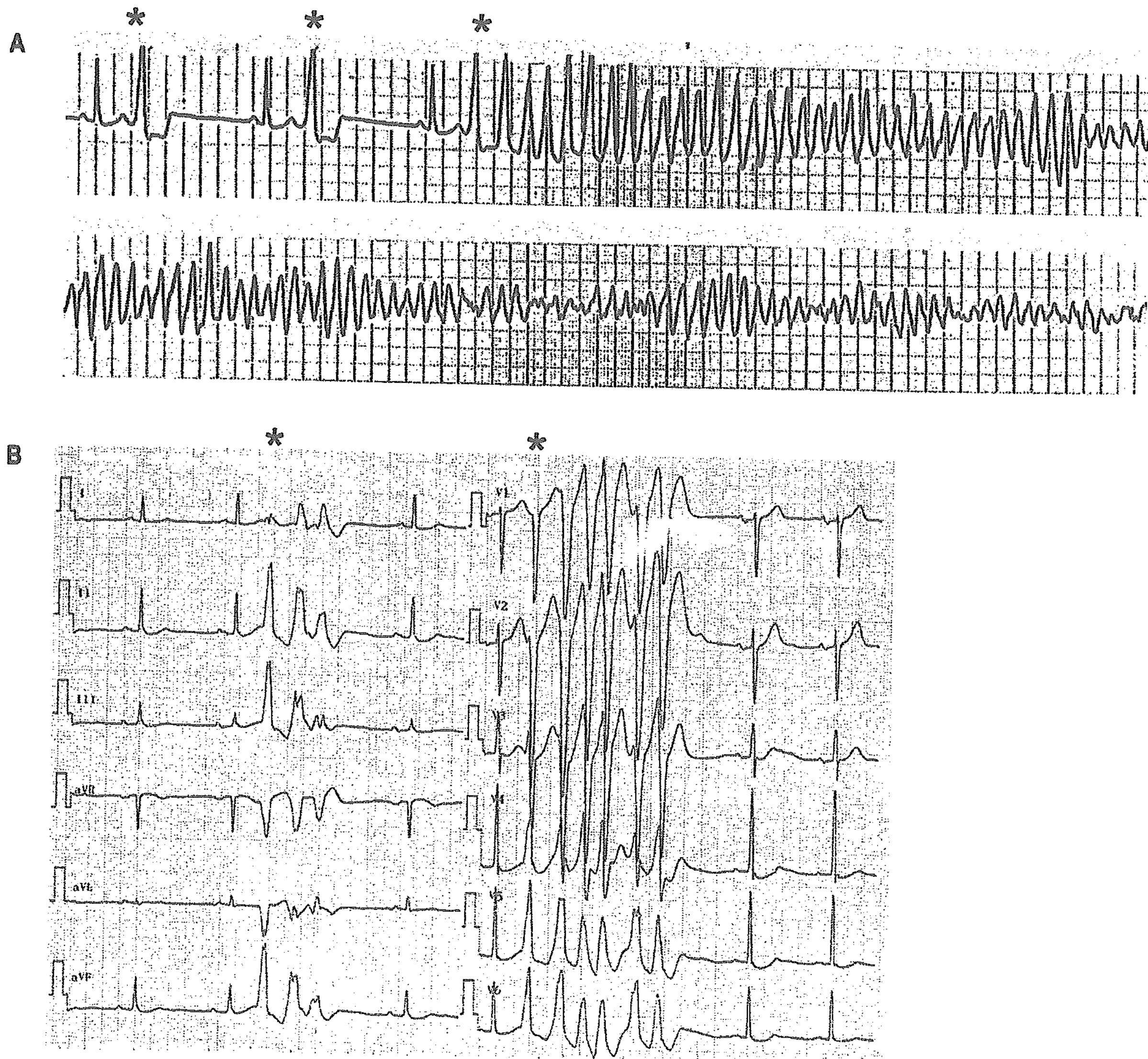

Figure 1. **(A)** Initiation of ventricular fibrillation (VF) recorded by a monitoring electrocardiogram in Patient #1. Note that the morphology of QRS complex of the initiating ventricular extrasystole (VE) was identical to that of preceding isolated premature ventricular contractions (*). **(B)** Non-sustained polymorphic ventricular tachycardia recorded in 12-lead electrocardiogram during hospitalization in Patient #8. The initiating VE showed left bundle branch morphology with inferior axis (*).

Table 1. Clinical Characteristics of the 16 Patients With the VF/PVT group

Patient No.	Age (yrs)	Gender	Spontaneous VF	Symptom	Holter ECG Findings: Isolated PVC (/day)	QT (ms)	CI (ms)	CL of PVT (ms)
1	43	F	+	Syncope	7,344	420	460	224
2	52	M	+	Syncope	26,828	420	410	240
3	41	F	+	Syncope	19,615	410	400	233
4	28	F	+	Syncope	7,518	430	440	216
5	48	F	+	Syncope	13,724	420	400	200
6	26	M	−	Syncope	NA	400	480	264
7	25	M	−	Pre-syncope	NA	420	580	310
8	49	M	−	Pre-syncope	41,192	400	390	273
9	43	M	−	Pre-syncope	2,479	380	360	248
10	41	M	−	Pre-syncope	13,819	360	320	242
11	38	F	−	Syncope	13,754	400	430	240
12	54	M	−	Syncope	9,700	380	360	220
13	41	F	−	Pre-syncope	12,681	390	360	268
14	25	F	−	Syncope	23,588	430	410	280
15	31	F	−	Syncope	38,061	380	350	227
16	34	F	−	Syncope	15,456	410	390	240
Mean ± SD	39 ± 10				17,554 ± 11,338	403 ± 21	409 ± 62	245 ± 28

Note that Patient #8 had a positive familial history of sudden cardiac death.

CI = coupling interval of the ventricular extrasystole; CL = cycle length; ECG = electrocardiogram; NA = not available; PVC = premature ventricular contraction; PVT = polymorphic ventricular tachycardia; QT = QT interval; VF = ventricular fibrillation; + = present; − = absent.

Electrophysiologic study and RFCA. As described previously (15), each patient underwent an electrophysiologic study (EPS) and RFCA in the fasting and non-sedated state after written informed consent was obtained. All drugs, including beta-blockers, were discontinued for at least five half-lives of each drug before the EPS. The 12-lead ECG of target VE that initiated spontaneous VF/PVT in the clinical course was also recorded in the EPS room before starting EPS and RFCA. If the target VE was not recorded under baseline conditions, injection of isoproterenol, epinephrine, or methoxamine with or without programmed ventricular stimulation was used to facilitate the induction of the initiating VE. We determined the provocated VE as target when the QRS morphology of the provocated VE was same as that of initiating VE recorded during hospitalization. Simultaneous 12-lead ECG and multiple intracardiac bipolar electrograms filtered at 30 to 500 Hz were recorded by a computerized electrophysiologic recording system (Bard Labsystem, CR Bard Inc., Billerica, Massachusetts) during EPS and RFCA. Stimuli were twice the diastolic threshold and 2 ms in duration. A deflectable 8-F quadripolar electrode catheter with conventional 4-mm distal electrode (EP Technologies, Sunnyvale, California) was used for mapping and RFCA with or without guidance by multielectrode basket catheter (Constellation, EP Technologies). Rapid burst pacing at multiple paced cycle lengths (pacing rate up to 250 beats/min) from right ventricular apex and the RVOT were performed in seven patients of the VF/PVT group before RFCA.

We performed RFCA by targeting the initiating VE. The optimal ablation site was determined by two methods: 1) endocardial activation mapping by identifying the site of the earliest activation during the target VE, and 2) pace mapping by comparing the 12-lead QRS morphology between the target VE and the paced beat during sinus rhythm. Radiofrequency energy was applied at the optimal site using a temperature control system with a target temperature set point of 60°C for 60 s. If the target VE was eliminated by energy delivery, three or four bonus applications were usually delivered around the most effective ablation site except in one patient. We tried to induce the target VE with the same interventions that provocated the target VE at the beginning of the EPS. If the premature ventricular contractions (PVCs) and/or ventricular tachycardia including the target VE were completely eliminated and were not induced at all, the RFCA was defined as successful. Partially successful ablation was defined when the target VEs were completely eliminated, but the other PVCs were induced and were not completely eliminated as a result. Failed ablation was defined when the target VEs were not eliminated completely.

Programmed electric stimulation was performed by up to triple extrastimuli mainly to confirm the effectiveness of RFCA as well as to induce VF in all 16 patients in the VF/PVT group after RFCA. We stopped extrastimuli at a coupling interval of 180 ms to avoid inducing non-specific VF.

Statistical analysis. Continuous variables were expressed as the group mean value ± SD and compared using unpaired t test. Qualitative variables were compared using Fisher exact test. A value of $p < 0.05$ was regarded as significant.

RESULTS

Clinical characteristics. Table 1 shows the clinical characteristics of the 16 patients with the VF/PVT group. Spontaneous episodes of VF were documented at rest during daytime in two patients and during nighttime in

Table 2. Comparison of the Clinical Parameters Between the VF/PVT Group and the RVOT-VT Group

	VF/PVT Group (n = 16)	RVOT-VT Group (n = 85)	p Value
Male	7/16 (44%)	25/85 (29%)	0.26
Age (yrs)	39 ± 10	43 ± 14	0.19
FH	1/16	1/85	0.29
Duration from onset of symptom to RFCA (months)	80 ± 103	69 ± 79	0.71
History of syncope	11/16 (69%)	15/85 (18%)	0.0001
Holter ECG findings			
Isolated PVC (/day)	17,554 ± 11,338	15,506 ± 16,053	0.58
CI of VE (ms)	409 ± 62	428 ± 65	0.27
QRS duration of VE (ms)	148 ± 8	142 ± 12	0.03
CL of VT (ms)	245 ± 28	328 ± 65	<0.0001

FH = family history of sudden death; RFCA = radiofrequency catheter ablation; RVOT = right ventricular outflow tract; VE = ventricular extrasystole; VT = ventricular tachycardia; other abbreviations as in Table 1.

three patients. Only one (Patient #8) of the 16 patients had a familial history of sudden cardiac death. Eleven of the 16 patients had prior episodes of syncope, and the remaining five patients had pre-syncope. Figure 1A shows the initiation of VF recorded by the monitoring ECG in Patient #1. The QT interval preceding VF was normal, and the coupling interval of the initiating VE was 460 ms. It is noteworthy that the morphology of QRS complex of the initiating VE was identical to that of the preceding isolated PVCs. In all patients, the corrected QT intervals preceding spontaneous VF/PVT were <440 ms. Holter recordings showed frequent isolated PVCs with the same QRS morphology as that of the initiating VE, and non-sustained PVT with short cycle length (mean of 245 ± 28 ms) in all 16 patients (Table 1). The coupling interval of VE was uniform in each patient and was not so short (mean of 409 ± 62 ms). Table 2 represents the comparison of the clinical parameters between the VF/PVT group and the RVOT-VT group. No significant difference was observed regarding gender, age, familial history of sudden death, and duration from onset of symptom to RFCA. However, prior episodes of syncope were more frequent in the VF/PVT group than in the RVOT-VT group (69% vs. 18%, $p = 0.0001$). In the Holter recordings, the frequency of isolated PVCs and the coupling intervals of the initiating VE were not different between the VF/PVT group and the RVOT-VT group. However, the cycle length of spontaneous non-sustained ventricular tachycardia was much shorter in the VF/PVT group than in the RVOT-VT group (245 ± 28 ms vs. 328 ± 65 ms, $p < 0.0001$).

Among the 16 patients in the VF/PVT group, 11 patients showed pre-syncope or syncope as a first symptom; however, the remaining 5 patients had only palpitation due to PVCs or monomorphic VT as a first symptom. Among five patients with spontaneous VF, three showed syncope as a first symptom, whereas two had only palpitation as a first symptom.

Electrophysiologic findings. Table 3 shows the electrophysiologic characteristics and RFCA parameters of the 16 patients in the VF/PVT group. The target VE occurred spontaneously in 11 patients and was induced by bolus injection of isoproterenol (1 μg) in three patients, epinephrine (5 μg) in one patient, and methoxamine (1 mg) in one patient. Endocardial mapping during sinus rhythm showed no abnormal electrograms, including fragmentations or delayed potentials, in any patients. His-ventricle intervals were <55 ms (mean of 42 ± 6 ms) in all patients. Figure 2 shows the polymorphic changes of the QRS complex during rapid pacing (pacing rate = 250 bpm) in Patient #3. These polymorphic morphologic changes were observed by the rapid pacing from origin of target VE in two patients (Patients #3 and #5) out of seven patients examined.

RFCA. Radiofrequency catheter ablation was performed at the site where the endocardial activation time during target VE was the earliest and the best pace mapping was obtained. Figure 3 represents the target VE (Fig. 3A), the pace mapping at the ablation site (Fig. 3B), simultaneous recording of surface ECG and endocardial electrograms during the target VE (Fig. 3C), and catheter position of the RFCA site (Fig. 3D) in Patient #2. The pace mapping demonstrated close concordance with the QRS morphology of the target VE in all leads. The bipolar endocardial electrogram of the mapping catheter, located in the septum of the RVOT, preceded the surface QRS onset of the target VE by 10 ms. The mean bipolar local activation time at the successful RFCA site was 17 ± 11 ms before the surface QRS onset (Table 3). The origin of the target VE, where the target VE disappeared or changed to the other VE with different QRS morphology by a single energy delivery, was in the septum of the RVOT in 13 patients and in the lateral freewall of the RVOT in three patients. After RFCA for initial target VE, the other VE with different QRS morphology appeared in 11 patients in whom multiple applications by mean of 9 ± 4 were added. Therefore, relatively large areas, approximately 2 to 4 cm in diameter, were presumably ablated in the 11 patients. Finally, RFCA was successful in 13 patients and partially successful in three patients by a mean of 9 ± 4 radiofrequency applications. Programmed electric stimulation after RFCA revealed that VF was induced by triple extrastimuli from the RVOT in only one patient (Patient #2), and non-sustained PVT was

Table 3. Electrophysiologic Characteristics and RFCA Parameters of the 16 Patients With the VF/PVT Group

Patient No.	Induction of Target VE	Origin of Target VE	ERP (ms)	EAT (ms)	Morphologic Change	No. of RF	Outcome	Induction of VF/PVT After RFCA	Advanced Treatment
1	Spontaneous	Sep	220	−10	−	1	Succ	−	−
2	Spontaneous	Sep	230	−10	−	5	Succ	VF (500/240/200/200)	ICD
3	Spontaneous	Sep	250	−20	−	8	Succ	−	−
4	Spontaneous	Sep	230	−50	+	11	Succ	−	−
5	Spontaneous	Sep	210	−8	+	15	Partial	PVT (500/240/220/190)	Beta-blocker
6	ISP	Sep	210	−20	+	12	Partial	−	Beta-blocker
7	Epi	Free	200	−18	+	4	Succ	−	−
8	Spontaneous	Sep	210	−12	+	10	Partial	−	Beta-blocker
9	Spontaneous	Free	200	−20	−	5	Succ	−	−
10	ISP	Free	220	−22	+	14	Succ	−	−
11	Spontaneous	Sep	200	−5	+	5	Succ	−	−
12	Me	Sep	220	−8	+	9	Succ	−	−
13	Spontaneous	Sep	200	−14	+	7	Succ	−	−
14	Spontaneous	Sep	210	−6	−	15	Succ	−	Beta-blocker
15	Spontaneous	Sep	210	−24	+	7	Succ	PVT (500/240/220/180)	−
16	Spontaneous	Sep	240	−26	+	8	Succ	−	−
Mean ± SD			216 ± 15	−17 ± 11		9 ± 4			

EAT = endocardial activation time (relative to QRS); Epi = epinephrine; ERP = effective refractory period; Free = freewall; ISP = isoproterenol; Me = methoxamine; Partial = partially successful ablation; PVT = polymorphic ventricular tachycardia; RF = radiofrequency applications; RFCA = radiofrequency catheter ablation; Sep = septum; Succ = successful ablation; VE = ventricular extrasystole; VF = ventricular fibrillation; + = present; − = absent.

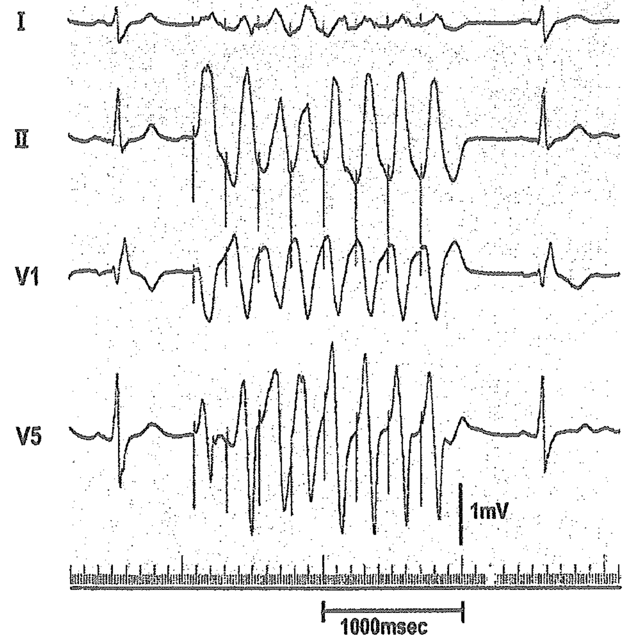

Figure 2. Polymorphic changes of the QRS complex on surface electrocardiogram leads I, II, V_1, and V_5 during rapid pacing in Patient #3. The morphologic changes were induced by rapid pacing from the origin of the target ventricular extrasystole.

induced in two patients (Patients #5 and #15) among the 16 patients.

Follow-up. One patient (patient #2) received an implantable cardioverter-defibrillator (ICD) because of induction of VF. Four patients received oral beta-blockers (3 for partially successful ablation and 1 for hypertension) as a therapeutic option. The remaining 11 patients were free from any advanced treatment, including antiarrhythmic drugs. There were no recurrences of episodes of syncope, VF, or cardiac arrest in any patients during follow-up of 54 ± 39 months.

DISCUSSION

Malignant entity of VF/PVT. Idiopathic ventricular tachycardias originating from the RVOT in patients without structural heart disease are considered benign, and RFCA has become an effective therapeutic option for these arrhythmias (8–12). However, a recent report (6) has shown that the malignant idiopathic VF may occasionally originate from the right ventricular outflow tract, the same site of origin of the "benign" RVOT. Moreover, several types of VF/PVT in patients without apparent heart disease were reported (14,16–23). We reported a malignant entity of VF/PVT initiated by the VE originated from the RVOT without structural heart disease in this study. In all patients, 12-lead ECG showed normal QT intervals at rest or just before and after episodes of VF and/or PVT (16–18). Neither ST-segment elevation nor right bundle branch block, most likely seen in Brugada syndrome, were recorded (14,19). The SAECG showed no late potential by which arrhythmogenic right ventricular cardiomyopathy/dysplasia

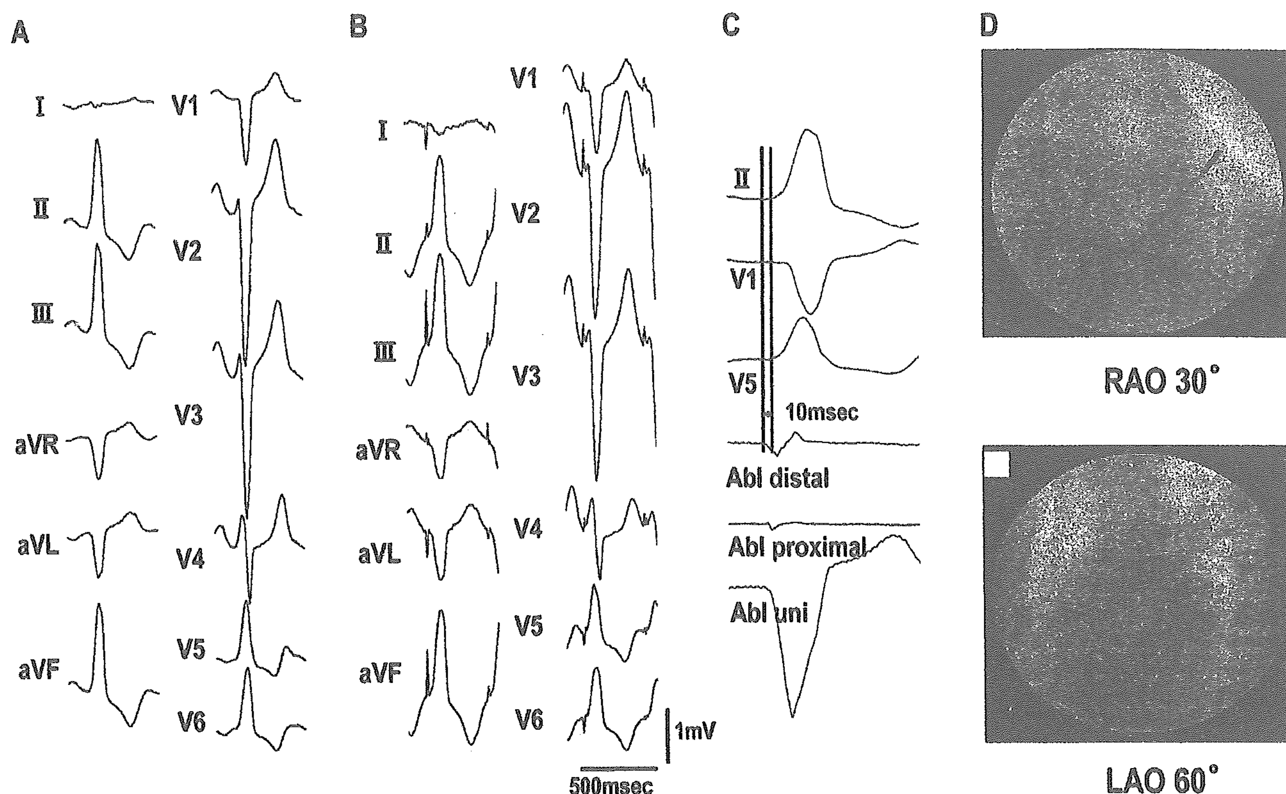

Figure 3. **(A)** Morphology of target ventricular extrasystole (VE) on 12-lead electrocardiogram (ECG); **(B)** Pace mapping at the ablation site; **(C)** Simultaneous recordings of surface ECG and endocardial electrograms during the target VE; **(D)** Catheter position of radiofrequency catheter ablation site in Patient #2. The pace mapping demonstrated close concordance with the QRS morphology of the target VE in all leads. The bipolar endocardial electrogram of the mapping catheter, which was located in the septum of the right ventricular outflow tract, preceded the surface QRS onset of the target VE by 10 ms. Abl = ablation catheter electrogram; LAO = left anterior oblique; RAO = right anterior oblique; uni = unipolar electrogram.

was characterized (20). The coupling intervals were not as short as those described in patients with short-coupled torsade de pointes (21) or idiopathic VF (22,23). Therefore, there are some differences in this entity compared with the prior types of idiopathic VF and/or PVT. We considered this entity to be a variant form of "benign" RVOT-VTs, but to have some difference in the clinical characteristics. The present data suggest that the 16 patients in the VF/PVT group showed prior episodes of syncope more frequently than the 85 patients in the RVOT-VT group, indicating the need of discrete follow-up in patients with prior episodes of syncope. On the other hand, 5 of the 16 patients in the VF/PVT group and two of the five patients with spontaneous VF had only palpitation as a first symptom. This finding suggests that some of patients initially diagnosed as "benign" RVOT-VT may become the patients with malignant entity of idiopathic VF/PVT, indicating that the need of careful follow up is required even in patients with benign RVOT-VT.

Possible mechanism of VF/PVT. It has been suggested that mechanism of idiopathic monomorphic VT arising from the RVOT is triggered activity (24,25). In this study, Holter recordings showed frequent isolated PVCs with the same morphology as that of the initiating VE. In all patients, the SAECG showed no late potential, and endocardial mapping represented no local abnormal electrograms, including fragmentations or delayed potentials. Programmed electrical stimulations induced VF in only one patient and non-sustained PVT in two patients among the 16 patients. In addition, rapid pacing from origin of target VE made the polymorphic morphologic changes in the QRS configuration in two out of seven patients, although the possibility that some beats were induced but are not captured by pacing could not be excluded completely. We speculate that the functional block and/or delayed conduction by rapid firing due to triggered activity or microreentry arising from a single focus led to chaotic ventricular conduction, so-called fibrillatory conduction, causing VF and/or PVT without organic delayed conduction zone. However, it is reasonable to say that rapid firing from close multiple foci one after another produces polymorphic morphologic changes in the QRS configuration in some cases. This is based on the observation that the other VE with different QRS morphology appeared after eliminating the initial target VE by RFCA in 11 patients. Although the initiating VE are likely to be generated from triggered activities, different mechanisms cannot be excluded.

We performed RFCA for the initiating VE with three or four bonus applications delivered around the most effective site. Some case reports showed successful RFCA for PVT