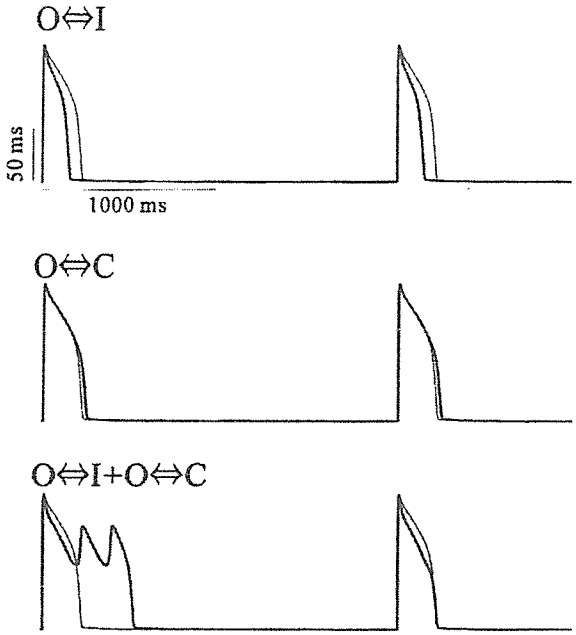

Fig 6. Individual effects of the 2 gating defects. One parameter change alone, which was associated with gain of function (O⇔I), shortened the action potential duration (APD), but failed to cause early after-depolarizations (EAD). The accelerated deactivation (C⇔O), which was associated with decreased tail currents, did not strongly influence APD, but only the condition of the combined gating defects (O⇔I+C⇔O) caused EAD. Wild-type, thin lines; N588K, thick lines.

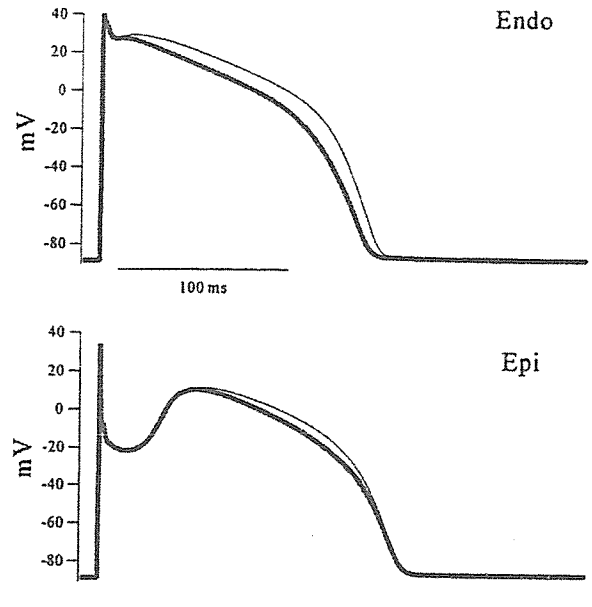

Fig 7. The contribution of transient potassium current (I_{to}) to phase I repolarization in epicardial (Epi) and endocardial (Endo) cells. Wild-type, thin lines; N588K, thick lines.

(Fig 5B). The inactivation of $I_{Ca(L)}$ currents involves voltage-dependent and calcium-dependent processes (Fig 5C and D). In the LRd model, they are represented by 2 gates, f and f_{Ca}, which are voltage- and calcium-dependent, respectively. The product of these 2 gates, $f \times f_{Ca}$, is an important parameter that indicates the recovery from inactivation of $I_{Ca(L)}$ and the availability of calcium channels for subsequent reactivation[9] The faster recovery from inactivation at a membrane potential in the range of $I_{Ca(L)}$ activation allows reactivation of $I_{Ca(L)}$, which being an inward current, results in secondary membrane depolarization to generate the EAD (Fig 5D).

Effects of Each Gating Defect for AP Simulations for LRd Model

We investigated how each of the 2 gating defects would affect the myocardial model (Fig 6). Unexpectedly, changes of 1 parameter alone, which was associated with gain of function or accelerated deactivation, could change the APD but failed to cause EAD. The increased transient rate from the O state to the closed state, which was associated with decreased tail currents, was strongly associated with EAD of the short-QT syndrome. Only the combination of the 2 gating defects could cause EAD (Fig 6). $I_{Ca(L)}$ reactivation also occurred only with the combination of the 2 gating defects.

Discussion

The KCNH2 potassium channel has a shaker-like tetrameric structure composed of homologous core units each containing 6 membrane-spanning segments. Co-assembly with the β-subunit MiRP1 (KCNE2) is required to fully reproduce the biophysical and pharmacological properties of the native I_{Kr}[14] KCNH2 has previously been linked to a decrease in outward repolarizing current responsible for the hereditary long-QT syndrome (LQT2)[15] and for acquired forms of long-QT syndrome (LQTS)[16] KCNH2 is also the primary target of class III antiarrhythmic agents, many of which contribute to generation of an acquired form of the LQTS. Brugada et al described the first mutation associated with the short-QT syndrome[3] The short-QT syndrome is genetically heterogeneous. It can also be caused by mutations in the KCNQ1 gene that encodes the KVLQT1 K^+ channel, which forms I_{Ks} in association with the β subunit IsK[17] and by mutations in the KCNJ2 gene that encodes the inwardly rectifying Kir2.1 channel[18]

We studied how gain of function of KCNH2 would cause life-threatening arrhythmia in the short-QT syndrome. The N588K mutation of KCNH2 was found to cause a remarkable gain of function in the I_{Kr} in short-QT syndrome[3] Based on the voltage-clamp experiments, the present simulation study revealed that N588K had 2 gating defects. Unexpectedly, 1 parameter change alone, which was associated with gain of function could shorten the QT intervals, but could not cause EAD by itself. The increased transition rate from the O state to a closed state (C1), which was associated with decreased tail currents, was strongly associated with EAD of the short-QT syndrome, and only the condition with both gating defects caused EAD. The previous report showed that the accelerated deactivation is related with LQTS[3] The accelerated deactivation for N588K mutant does not decrease APD, but increases APD. However, the balance between impaired inactivation and the accelerated deactivation would determine APD, and gain of function in the I_{Kr} might be a strong determinant of APD in the case of N588K. McPate et al and Cordeiro et al reported that N588K channels alter the ion selectivity[11,12] Reversal potentials for the N588K mutant are significantly higher. We also studied how the ion selectivity could affect EAD for

N588K channels. Higher reversal potential could easily cause EAD for N588K channels with double gating defects (data not shown).

$I_{Ca(L)}$ reactivation seems to be a universal mechanism of EAD from plateau potentials. It is well known that EAD cause ventricular tachycardia in the LQTS[4,9] Prolongation of the post-pause AP plateau because of a smaller I_{Ks} or I_{Kr} and an enhanced inward I_{NaCa}, on the background of the LQTS effects, provides time for the recovery and reactivation of $I_{Ca(L)}$, which generates the depolarizing charge for EAD formation. However, the faster recovery from inactivation in the short-QT syndrome allows for the reactivation of $I_{Ca(L)}$.

The group of Antzelevitch presented the first experimental evidence of the role of transmural dispersion of repolarization (TDR) in arrhythmogenesis associated with short-QT intervals in the ECG[6] Pinacidil was used to mimic a gain of function of a potassium current in the canine arterially perfused wedge preparation. Pinacidile activates adenosine triphosphate-sensitive potassium currents (I_{K-ATP}), leading to an abbreviation of APD![19,20] In the wedge preparation, pinacidil abbreviated the QT interval, leading to a heterogeneous abbreviation of the APD of the 3 principal cell types spanning the ventricular wall. The pinacidil model of the short-QT syndrome, although mechanistically related, is phenotypically different from the clinical syndrome caused by a gain of function of HERG.

The computer simulation is a good tool to predict APD of genetic arrhythmogenic disorders, such as the LQTS and Brugada syndrome[4,5,7] We could successfully reproduce these alterations of gating properties in the short-QT syndrome by changing 2 parameters in the Markov model of the KCNH2. When the I_{Kr} channels were all N588K mutants, APD of each wall layer was smaller than that of WT. The TDR_{max} of N588K was also smaller than WT at any BCL (data not shown). These results are opposed to the wedge models. However, Brugada et al showed that the mid- and endocardial regions of the ventricle would repolarize more rapidly than the epicardium, because currents mediated by mutated HERG channels electrontically follow the membrane potential. The presence of a notch during phase I therefore lowers the amplitude of the N588K currents in the epicardium vs endocardium and increase TDR. We tested this hypothesis by modifying the LRd model to take into account the contribution of transient potassium current (I_{to}) to phase I repolarization of the epicardium, according to modifications published earlier by Dumaine et al[21] Although mutant APD for endocardial cells shorten in the presence of I_{to}, shortening of the APD for epicardial cells was slight, and these results lead to increase TDR (Fig 7). Cordeiro et al recently reported that the dispersion of repolarization between the Purkinje fibers and ventricles induced by N588K might create a substrate for premature re-excitation of the endocardium![11] In the short-QT syndrome, ventricular tachycardia might occur in the substrate of both EAD in M cells and the dispersion of repolarization between the Purkinje fibers and ventricles. We might need to analyze the transmural AP mapping by applying a validated optical mapping technique![22]

Clinically, we have not clearly understood what triggers ventricular tachycardia in the short-QT syndrome unlike the LQTS[23,24] because adequate genotype-phenotype correlation data are not yet available for the short-QT syndrome. A latest report shows that shortening of the QT interval is prominent at lower heart rates[25] Therefore, we speculate that a potential trigger might occur in ventricular tachyarrhythmias at rest and during sleep in patients with a short-QT syndrome.

In summary, a part of the short-QT syndrome has been linked to gain of function mutation of KCNH2. Although the gain of function for KCNH2 causes shortened APD of the short-QT syndrome, arrhythmogenesis might be associated not only with gain of function, but also with accelerated deactivation of HERG.

Limitations

First, the simulation studies are generally simplified. However, the simulation studies lead to understand complex and interactive phenomena, and relate them to membrane ionic currents and to dynamic changes in the intracellular ionic environment[7] Second, the simulation studies were conducted in isolated cell models. The ionic mechanism in the single cell helps us to understand the basis of its behavior in the multicellular tissue with complex interactions. In the intact myocardium, cells are interconnected through gap-junctions and we need to study its ionic mechanism in the multicellular tissue level![26]

Acknowledgment

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Circ J 2006; **70:** 933–935

Novel Mutation of Plakophilin-2 Associated With Arrhythmogenic Right Ventricular Cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disease characterized by dilatation and akinesis of the right ventricle, and causes life-threatening ventricular arrhythmia. Mutations of plakophilin-2 (PKP2) have recently been identified as one causative abnormality in ARVC. A case of ARVC with a mutation of PKP2 is reported here. Direct sequencing of the patient's DNA revealed an insertion mutation in exon 8 of PKP2 (1728_1729insGATG). The mutation caused the frameshift and the premature termination of translation (R577DfsX5). This is the first case report of PKP2 mutation found in Japanese ARVC patients. (*Circ J* 2006; **70:** 933–935)

Key Words: Arrhythmogenic right ventricular cardiomyopathy; Desmosome; Genetic analysis; Plakophilin-2

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disease characterized by dilatation and akinesis of the right ventricle that causes life-threatening ventricular arrhythmias. A characteristic pathological finding is a progressive fibro-fatty replacement of the right ventricular myocardium[1] About 30–50% of the cases of ARVC are inherited, and heterozygous mutations of ryanodine receptor-2 (RYR2)[2,3] and plakophilin-2 (PKP2) have been reported in familial ARVC[4] PKP2 has essential roles in the formation of desmosome and heart development[4,5] In this short report, we presents the first Japanese ARVC patient in whom a novel mutation of PKP2 was identified.

Case Report

A 30-year-old male was referred to hospital due to recurrent faintness. Physical examination revealed a heart rate of 50 beats/min and blood pressure of 114/60 mmHg. No heart murmur was heard and there were no signs of left or right ventricular failure. The chest X-ray revealed cardiomegaly with increased cardiothoracic ratio (58%). The blood chemistry showed no abnormalities. His resting electrocardiography (Fig 1A) showed T wave inversion in leads V_{1-4}, without right bundle branch block. Sustained ventricular tachycardia of the left bundle branch block morphology with an inferior axis was recorded during the period of faintness (Fig 1B). Signal-averaged ECG recordings showed positive late potentials according to the following criteria: filtered QRS duration 181 ms (>130 ms), duration of low amplitude signals <40 μV of the terminal QRS complex (LAS40) 102 ms (>40 ms), and root mean square voltage of the last 40 ms of the QRS complex (RMS40) 2.0 μV (<15 μV) (Fig 1C)[6] Echocardiography revealed an enlarged, hypokinetic right ventricle with a paper-thin free wall (Fig 2). Contrast-enhanced computed tomography demonstrated the dilated right ventricle and the presence of epicardial and intramyocardial fat deposits in the right ventricle (Fig 3). His aunt died suddenly in her fifties. Accordingly, the patient was diagnosed as ARVC and gave an informed consent for the genetic analysis.

Genetic Analysis

The patient's genomic DNAs were extracted from peripheral blood using standard methods after obtaining informed consent. The institutional review boards approved the protocols. All exons of PKP2[4] and some parts of RYR2 (exons 8–16, 44–49, 83, 84, 87–89, 91–105), were examined using denaturing high performance liquid chromatography (DHPLC; WAVE system, Transgenomic Inc, Omaha, USA)[3] A mixture of 15 μl of each DNA sample from the patient and from a normal control was heated for 5 min at 95°C, and then cooled down to various temperatures depending on the primer setting. The resultant chromatograms were compared for variation in shape or retention time. All variants identified by the DHPLC scanning were examined by direct sequencing using ABI PRISM 310 DNA Sequencer (Perkin Elmer, Foster City, USA).

We found no mutation in 35 exons of RYR2 gene, which are thought to be hot regions for mutations[3] In the exon 8 of PKP2 gene, however, the chromatogram of DHPLC showed a variant elution pattern in the patient's DNA (Fig 4). Direct sequencing showed overlapping figures due to an insertion mutation, causing the frameshift (1728_1729insGATG) (Fig 5A). This mutation caused a premature termination of translation at the codon 582 (R577DfsX5) (Fig 5B).

Discussion

Plakophilin (PKP) is an essential protein forming the desmosomal complex[4,5] Type 2 PKP (PKP2) encoded by *Pkp2* is the main isoform in cardiomyocytes. Grossmann et al reported that the ablation of mouse *Pkp2* resulted in the

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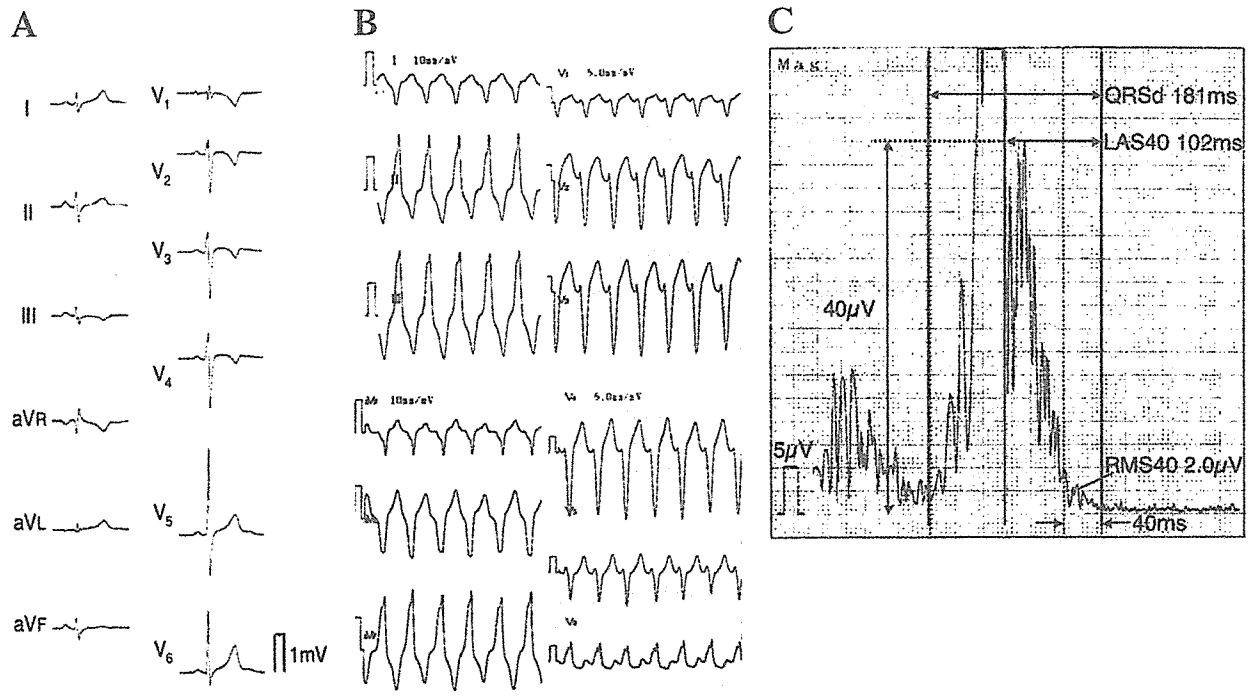

Fig 1. (A) Resting 12-lead electrocardiograms showing the T wave inversion in chest leads V_{1-4}, in the absence of right bundle branch block. (B) Sustained ventricular tachycardia of left bundle branch block morphology with an inferior axis was recorded during the period of faintness. (C) Signal-averaged ECG recordings.

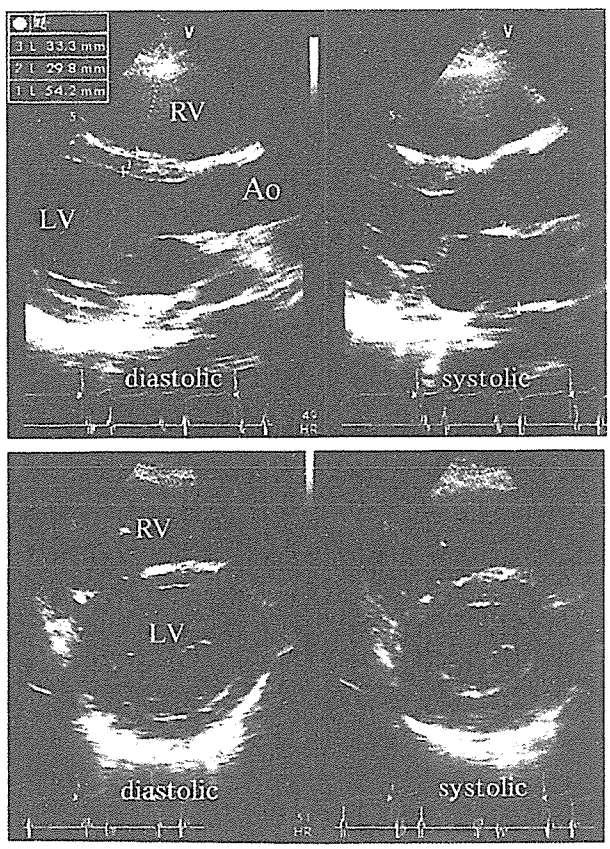

Fig 2. Echocardiography revealed an enlarged, hypokinetic right ventricle (RV) with a paper-thin free wall. LV, left ventricle; Ao, ascending aorta.

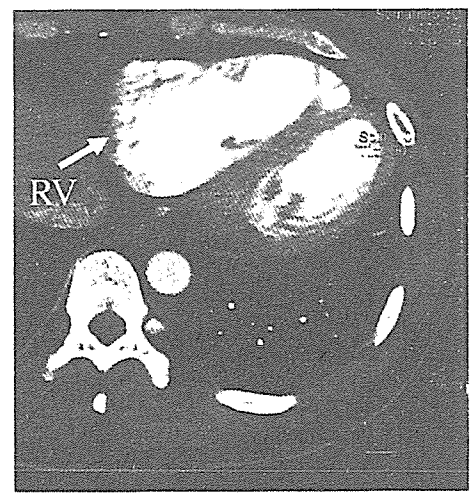

Fig 3. Computed tomography with contrast demonstrated the dilated right ventricle (RV) and the presence of epicardial and intramyocardial fat deposits in the RV.

lethal defect of heart morphogenesis at embryonic day 10.75[5] Genetically-engineered transgenic mice lacking *Pkp2* have been shown to disrupt the cell–cell contacts of adjacent cardiomyocytes, leading to right ventricular dilatation similar to that observed in human ARVC. Besides PKP2, the disruption of desmosomal proteins such as plakoglobin and desmoplakin has been identified in inherited forms of ARVC[7,8] Pashmforoush et al reported that disruption of the gene encoding α-actinin-associated LIM protein in mice caused dilatation and dysfunction of the right ventricle in utero[9] Therefore, Gerull et al stated that ARVC might be considered a desmosome disease[4] As shown in

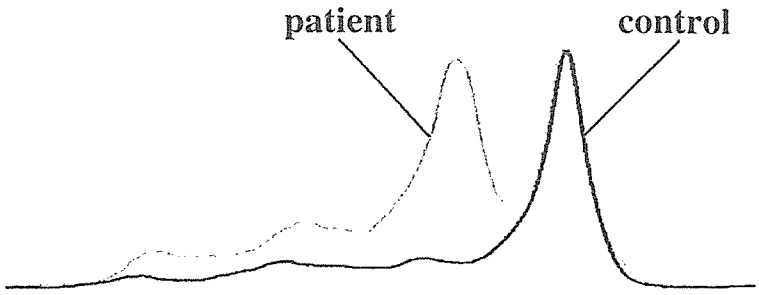

Fig 4. In exon 8 of the plakophilin-2 gene, denaturing high performance liquid chromatography chromatogram representing variant elution pattern of patient's DNA.

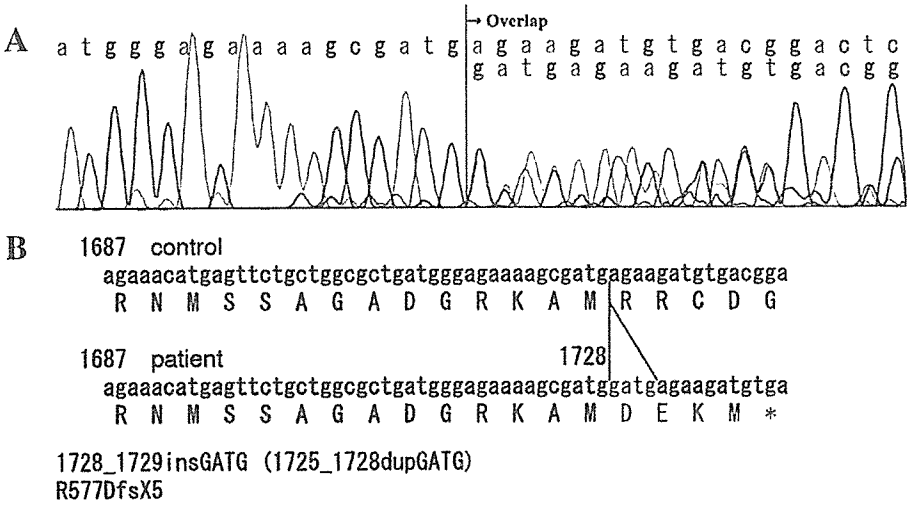

Fig 5. (A) Direct sequencing revealed insertion mutation 1728_1729ins GATG (1725_1728dupGATG). (B) Alignment of cDNA in the vicinity of codon 1728. The insertion mutation causes the frame shift and the premature termination of translation (R577DfsX5 indicated by asterisk).

Pkp2 knock-out mice, disruption of desmosome leads to the loss of cell to cell connection, which in turn causes replacement of the myocardium with fibro-fatty tissue and thereby causes a regional conduction delay.[5] Such histological changes may cause the positive late potential (Fig 1C).

In the present study, DHPLC enabled us to examine a number of DNA samples at the same time and save time detecting single base substitutions of DNA fragments. Yamanoshita et al reported that DHPLC is superior to single-strand conformational polymorphism (SSCP) in screening for mutations in terms of sensitivity.[10] However, DHPLC may not be 100% effective in the detection of mutations.[11] Moreover, we did not search for RYR2 mutations out of the regions known as hot sites.[3] Also, the possibility that there might be mutations within regulatory regions or intronic sequences important for splicing or transcription cannot be excluded.

In summary, we found a novel mutation of PKP2 associated with ARVC by using a screening technique of DHPLC and direct sequencing. This is the first case of PKP2 mutation found in a Japanese ARVC patient.

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Gender and Age Effects on Ventricular Repolarization Abnormality in Japanese General Carriers of a G643S Common Single Nucleotide Polymorphism for the *KCNQ1* Gene

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Background The *KCNQ1* single nucleotide polymorphism (SNP), G643S, is known to be associated with secondary long QT syndrome (LQTS) and to cause a mild reduction in *KCNQ1* current. However, the precise incidence and its association with QT intervals remain unknown in the greater cohort of the population in Japan.
Methods and Results The genotype was screened at codon 643 of *KCNQ1* in 992 residents of a farming community. Eighty-eight individuals (female/male=52/36, 8.9%) were found to have a heterozygous G643S SNP. Matching both gender and age, we randomly selected 243 control (G643G) cases and compared the electrocardiogram parameters in both groups; QT, QTf (QT corrected by Fridericia's formula) intervals, the peak and the end of the T wave (Tpe) interval, and the Tpe/QT ratio. The latter 2 reflect the transmural dispersion of ventricular repolarization (TDR). In G643S carriers, both Tpe and Tpe/QT were significantly longer than in non-carriers, without significant QT prolongation. Both genders showed a tendency for an increase in QTf with aging. In females, both Tpe and Tpe/QT showed a similar significant increase with age, which was not observed in males.
Conclusions In elderly females, G643S might be an independent risk factor for secondary LQTS by causing a greater TDR. (*Circ J* 2006; **70:** 645–650)

Key Words: Electrocardiography; *KCNQ1*; Secondary LQTS; Single nucleotide polymorphism; Transmural dispersion of repolarization

A potassium channel gene, *KCNQ1*, encodes the α-subunit of the slow delayed rectifier K (I_{Ks}) channel with accessory protein, MinK (coded by *KCNE1*)[1] Since the first report on the relationship between *KCNQ1* mutations and congenital long QT syndrome (LQTS)[2] more than 120 genetic variations have been identified in *KCNQ1*.[3,4] Among these genetic variations, a transposition of guanine to adenine at the 1727 nucleotide, causing G643S, was found to be a single nucleotide polymorphism (SNP) in ~11% of the Japanese general population and has been shown to cause a very mild reduction in I_{Ks} reconstituted in the mammalian cell line.[5,6] This SNP, therefore, appeared to be a risk factor predisposing the secondary LQTS. The relationship between the G643S genotype and the phenotype in regard to electrocardiogram (ECG), however, remains unclear. In the present study, we screened the SNP among 992 habitants in a farming community (Shigaraki) near Kyoto, Japan, and examined the characteristics of the ECG parameters in both SNP-positive and negative individuals.

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More recently, in addition to the absolute QT interval, transmural dispersion of repolarization (TDR) has been evaluated by measuring the interval between the peak and the end of the T wave (Tpe) and the Tpe/QT ratio.[7–10] These TDR parameters have drawn our attention in regard to the occurrence of torsades de pointes (TdP) in LQTS.[11–14] Therefore, in the present study, we measured these TDR indicators along with conventional QT intervals in genotyped G643S carriers and compared these results with those in non-carriers. The incidence of cardiac events in patients with congenital LQTS has been known to vary, dependent on both gender and age. For example, young male and elderly female LQT1 patients have a higher risk of having cardiac events.[15,16] This is also true for the acquired type of LQTS; victims of TdP tend to be elderly females.[6,17–18] Taken together, gender and age could largely influence the phenotype induced by a genetic variant, especially in cases where the functional change resulting from the variant was subtle.[19] We therefore analyzed the ECG parameters of G643S carriers and non-carriers by dividing the subjects of the study into several groups depending on their gender and age.

Methods

DNA Isolation and Genotyping

Among 2,902 individuals who entered the Shigaraki Study,[20] 992 were enrolled to the present study; others were

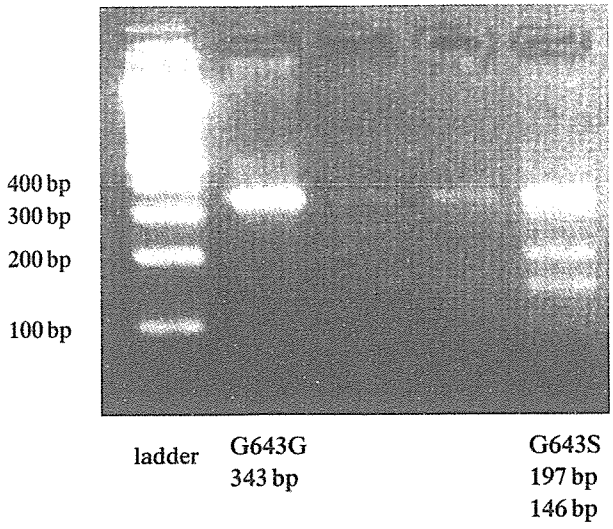

Fig 1. Electrophoresis of G643G and G643S polymerase chain reaction (PCR) products. The PCR products were electrophoresed on a 2.0% Agarose gel (Nippon Gene, Japan), and observed for a 343 base pair (bp) fragment (*KCNQ1* G643G genotype), and both 146 bp and 197 bp fragments (*KCNQ1* G643S genotype).

excluded from the analysis because of current drug intake, diabetes mellitus and a history of cardiac diseases. Genomic DNA was isolated from peripheral leukocytes, and the *KCNQ1* genotype at codon 643 was determined by using a restriction enzyme. Briefly, for the genomic DNA from each individual (extracted by using a DNA extraction Kit; Wako, Japan), a set of primers encompassing the DNA fragment containing codon 643 was used for polymerase chain reaction (PCR) (sense: 5'-ACTCATCACCGACATG-CTTCACCAGCT, anti-sense: 5'-CTTTTAGGAGGTGGC-CTCCTTCAGA). The presence of a SNP was determined by finding whether the restriction endonuclease, Pst-I (Takara, Japan), cleaved the PCR product (Fig 1). Finally, the correlation between the electrophoresis pattern and the genotype was confirmed by using a direct sequencing method (ABI PRISM 310 Genetic Analyzer, Parkin-Elmer, USA).

Case and Control Definition

Among the 992 participants, 88 had the G643S SNP (36 males, 52 females) and 904 had the G643G wild genotype (344 males, 560 females). Thus, the heterozygous genotype was estimated to be 8.9% (88 out of 992). In the 88 SNP carriers, 7 subjects, 2 males and 5 females, showed abnormal 12-lead ECG at rest (eg, ischemic change, conduction abnormalities, atrial fibrillation, non-specific ST change, or left ventricular hypertrophy) and were excluded from the analysis. Finally, the SNP group was comprised of 81 individuals (34 males, 47 females). For each individual G643S carrier, 3 control cases were randomly drawn from G643G carriers by matching both gender and age (102 males, 141 females). None of these control cases showed abnormal ECG. The protocol used in the present study was approved by the Institutional Review Board of Shiga University of Medical Science (Nos. 11–15, 1999).

ECG Measurements

All ECG parameters were measured manually (Fig 2). QT was defined as the interval between the QRS onset and the end of the T wave, at the point where the isoelectric line intersected a tangential line drawn on the maximal downslope of the positive T wave. Q-Tpeak (QTp) was defined as the interval between the QRS onset and the peak of the T wave. Then, the interval between the peak and the end of the T wave (Tpe) was calculated as QT minus QTp, and Tpe/QT was calculated as the relative Tpe interval divided by the QT interval. The interval of the Tpe has been shown to reflect the TDR[7–9] We examined the characteristics of the ECG parameters using the V_5 lead, because it is known that the unipolar lead reflects the local electric potential gradient of the free wall at the left ventricle[7,8] Measurements were performed as the mean of approximately 3 consecutive beats by 2 investigators who were unaware of the subject's status. There were no significant differences in the measured numerical data obtained by the 2 investigators.

Statistical Analysis

Data are presented as mean ± SD. Multivariate regression analysis was used for the comparison of each ECG parameter over 4 effects (genotype, gender, heart rate (HR), and age; Table 1). The non-paired 1-tail Student's t-test was used to compare the unpaired parameters (HR, age, and ECG parameters) between the different groups (Tables 2–4;

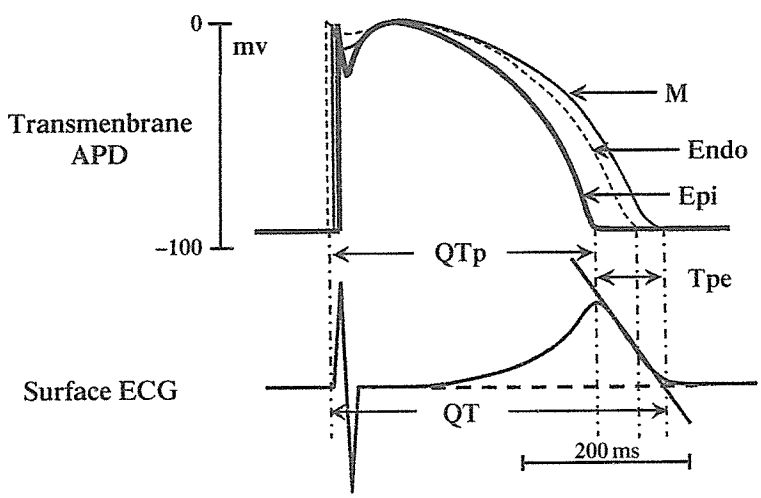

Fig 2. Variety of action potential duration (APD) in each myocardium layers and the electrocardiogram (ECG) repolarization parameters. Endo, the APD of the endocardial layer; M, the APD of the subendocardial layer; Epi, the APD of the epicardial layer; QTp, the interval between the Q-point and the T-wave peak; Tpe, QT interval − Q-Tpeak interval.

Table 1 Multivariate Regression Analysis of Repolarization Parameters for Each Category

Category	QT		QTc		QTf		Tpe		Tpe/QT	
	F ratio	p value	F ratio	p value	F ratio	p value	F ratio	p value	F ratio	p value
Genotype	0.010	NS	0.263	NS	0.181	NS	12.760	<0.001	12.465	<0.001
Gender	4.779	<0.05	19.276	<0.001	5.106	<0.05	1.217	NS	3.777	0.052
HR	293.8	<0.0001	86.146	<0.001	0.218	NS	0.044	NS	28.209	<0.0001
Age	5.842	<0.05	9.197	<0.01	11.610	<0.001	6.870	<0.01	2.527	NS

QTc, $QT/RR^{1/2}$; QTf, $QT/RR^{1/3}$; Tpe, QT interval–Q-T peak interval; HR, heart rate.
The data were derived from 136 males and 188 females, Shigaraki town, Shiga, Japan, 1999.

Table 2 Characteristic Status in Each Genotype

Genotype	Age (years)	HR (beats/min)	QT (ms)	QTc (ms)	QTf (ms)	QTp (ms)	Tpe (ms)	Tpe/QT
G643S (n=81)	54.0±14.5	64.3±9.4	394.1±26.7	405.3±22.3	401.3±19.4	306.1±28.1	88.0±12.3	0.224±0.034
G643G (n=243)	54.4±15.5	63.7±8.9	394.3±26.8	404.1±22.8	400.6±20.2	312.3±27.4	82.0±13.3	0.209±0.034
p value	NS	NS	NS	NS	NS	NS	p<0.0001	p<0.0001

Data are mean±SD. Probability values between G643S carriers and non-carriers.
QTp, Q-T peak interval; G643S, carriers; G643G, non-carriers. Other abbreviations see in Table 1.
The data were derived from 136 males and 188 females, Shigaraki town, Shiga, Japan, 1999.

Table 3 Characteristic Status in Each Gender-Genotype

	Age (years)	HR (beats/min)	QT (ms)	QTc (ms)	QTf (ms)	Tpe (ms)	Tpe/QT (ms)
G643S carriers							
Male (n=34)	53.2±16.6	60.1±9.2	397.1±28.3	394.6±17.9	395.2±17.1	89.2±9.3	0.226±0.029
Female (n=47)	54.5±13.1	67.1±8.5	392.0±25.7	412.7±22.1	405.5±20.0	87.2±14.0	0.223±0.037
P_1	NS	p<0.001	NS	p<0.001	p<0.05	NS	NS
G643G non-carriers							
Male (n=102)	53.3±16.1	61.2±9.4	397.3±24.8	398.7±21.4	398.0±17.3	81.1±12.0	0.204±0.031
Female (n=141)	54.9±15.3	65.7±7.9	391.7±27.8	408.0±22.7	402.3±21.7	83.1±14.1	0.212±0.035
P_2	NS	p<0.001	NS	p<0.05	NS	NS	p<0.05
P_3/P_4	NS/NS	NS/NS	NS/NS	NS/NS	NS/NS	p<0.001/NS	p<0.001/NS

Data are mean±SD. P_1 and P_2 are the probability values, male vs female, in each genotype, respectively; P_3/P_4, G643S vs G643G in male/in female, respectively.
Abbreviations see in Table 1.
The data were derived from 136 males and 188 females, Shigaraki town, Shiga, Japan, 1999.

Table 4 Demography and Characteristic Status of Each Gender

	Age subset (years)					
	<31	31–40	41–50	51–50	61–70	>70
Number						
Male	16	24	24	16	40	16
Female	20	16	36	36	52	28
Mean age (years)						
Male	25.0±4.0	36.8±2.8	47.0±2.6	56.2±2.6	65.8±2.5	73.7±3.9
Female	27.9±3.0§	35.1±2.2*	46.5±3.3*	56.9±2.6*	65.1±2.6*	74.4±3.3*
HR (beats/min)						
Male	62.3±12.2	60.1±8.5	61.3±9.3	63.6±8.9	59.1±8.6	62.2±10/0
Female	66.4±6.6*	66.9±10.3§	67.0±7.7§	65.0±8.6*	65.5±8.1§	66.7±7.9*

*Data are mean±SD. §p<0.05; *p=NS vs female in each age subset.*

Figs 3–5). Univariate regression analysis was used for comparisons of the repolarization parameters and age (Fig 5). A probability value of <0.05 was considered significant.

Results

Characteristics of ECG Parameters: Genotype Effects on TDR

For the 992 total cases, the resting HR ranged from 41 to 91 beats/min., and the age from 19.1 to 85.4 years. There was no significant correlation between HR and age. There was no subject having a QTc >480 ms[21,22] T-waves of G643S carriers showed a broad-based pattern, and there was no case with obvious bifid T-waves among all carriers and control cases.

Table 1 summarizes the correlation between the ECG parameters and genotype (G643S or G643G), gender, HR or age. QT, QTc (Bazett's correction formula=$QT/RR^{1/2}$),

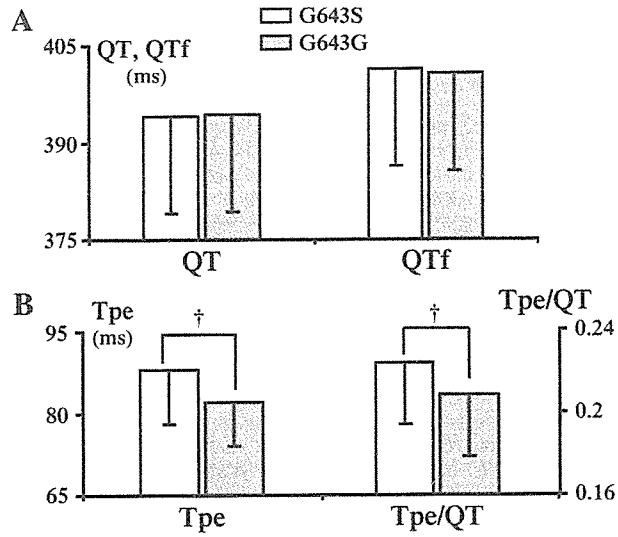

Fig 3. Two genotypes and electrocardiogram repolarization parameters. The relationship between the intervals of each ventricular repolarization; QT, QTf (A), Tpe, and Tpe/QT (B), and the genotype are presented for G643S carriers (blank portion) and G643G non-carriers (shaded portion). QTf indicated $QT/RR^{1/3}$; Tpe, QT interval−Q-Tpeak interval (Q-Tpeak, the interval between the Q-point and the T-wave peak); Tpe/QT, the relative value of Tpe divided by QT; G643S, carriers; G643G, non-carriers. Data are presented as mean± SD. †p<0.0001.

and Tpe/QT showed a strong correlation with HR, but Tpe remained independent of HR.[23] Therefore, absolute Tpe values were adopted for examination, without correction by HR, and Tpe/QT was used as the relative fractional proportion to the QT interval. The present study population showed a wide range of HR, and it is known that QTc is strongly affected by HR, and that HR is strongly affected by gender.[24,25] Indeed, in the present study cohort, HR affected QTc but not Fridericia's correction formula ($QTf = QT/RR^{1/3}$), whereas gender affected both QT corrections (Table 1). It could be said that QTf had a net relationship with gender, irrespective of HR. We therefore used QTf in the following analyses.

The genotype at codon 643 of *KCNQ1* showed a significant correlation with 2 repolarization parameters, Tpe and Tpe/QT (Table 1). Fig 3 and Table 2 summarize the comparison of the ECG parameters between the 2 genotypes. Tpe and Tpe/QT were significantly larger in G643S than in G643G carriers, although there were no significant differences of QT or QTf intervals between the 2 genotypes.

Different Genotype Effects on Repolarization Parameters in Each Gender

The QT and QTf values showed a significant correlation with gender and age in all of the study population (Table 1). We divided the 2 genotype groups by gender, resulting in 4 subsets. Table 3 and Fig 4 summarize the characteristics of each repolarization parameter in these 4 subsets. In both genotype groups, the mean QT interval was longer in males than in females. This was because HR was significantly lower in males (Table 3), and there was HR-dependent prolongation of the QT interval.[26] In contrast, the mean QTf interval was longer in females (Table 3), and the female G643S subset showed a significantly longer QTf interval than the male G643S subset (p=0.02). There was no difference in Tpe and Tpe/QT between the 2 genders. However, between the 2 genotypes, Tpe and Tpe/QT values were longer in the G643S group than in the control. This was significant only between the 2 male groups (p<0.02) (Figs 4B,C).

TDR Increases With Age, Especially in Female Groups

In our initial analyses of the correlation between categorical and ECG parameters in 324 individuals (Table 1), it was found that age was significantly correlated with the ECG parameters. Fig 5 shows a summary of the age-dependent changes in 4 ECG parameters in the 2 gender groups, and the demography and characteristics of each gender are presented in Table 4. QTf, Tpe and Tpe/QT showed a tendency to increase prominently with age in females. Similar

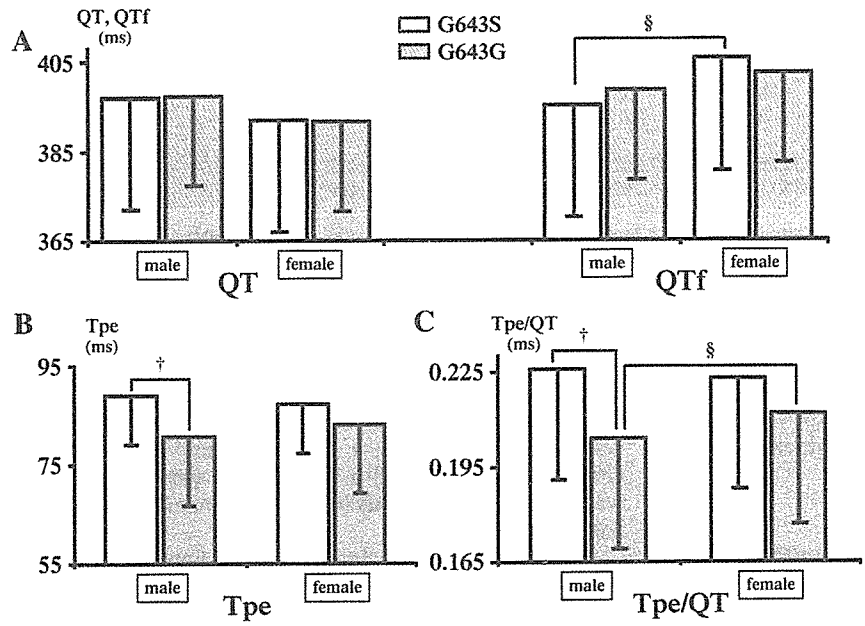

Fig 4. Gender-dependent analyses on genotype-electocardiogram (ECG) parameter relationship. The relationship between the intervals of each ventricular repolarization; QT, QTf (A), Tpe (B), and Tpe/QT (C), and the genotype in each gender are presented for G643S carriers (blank portion) and G643G non-carriers (shaded portion). QTf indicated $QT/RR^{1/3}$; Tpe, QT interval−Q-Tpeak interval (Q-Tpeak, interval between the Q-point and the T-wave peak); Tpe/QT, Tpe divided by QT; G643S, carriers; G643G, non-carriers. Data are presented as mean± SD. §p<0.05; †p<0.0001.

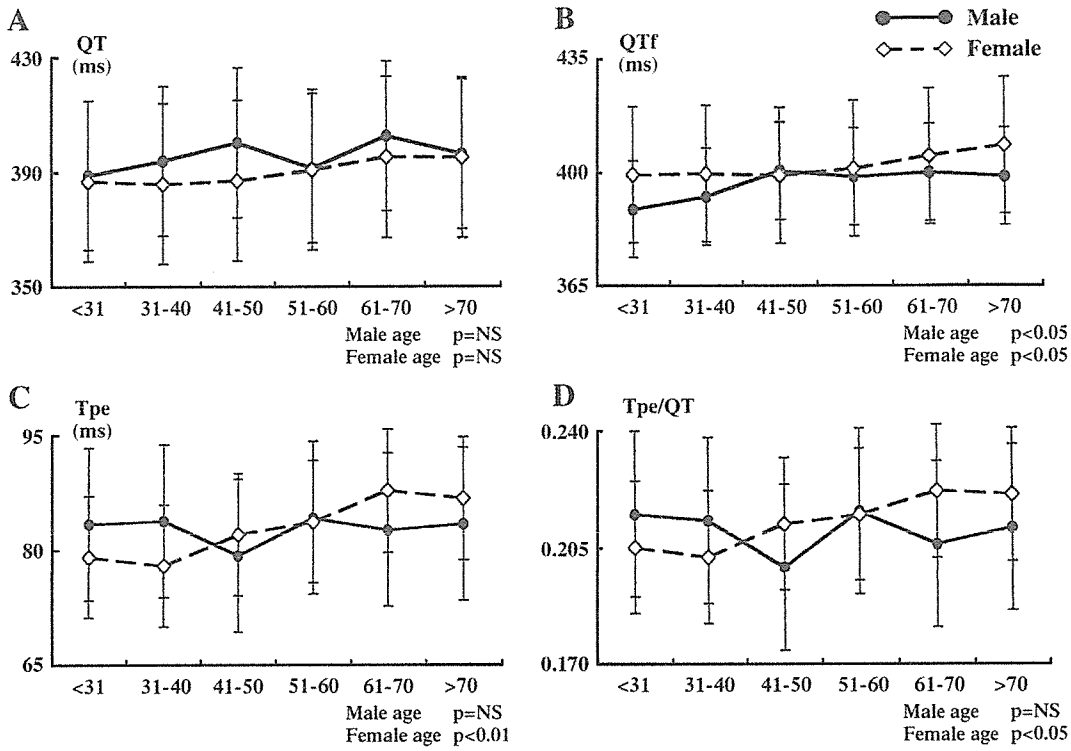

Fig 5. Age-dependent analyses on the electrocardiogram (ECG) parameters. The correlations of the intervals of each ventricular repolarization; QT (A), QTf (B), Tpe (C), and Tpe/QT (D), and ages are presented for the male carriers (solid line and ●) and for the male non-carriers (dotted line and ◇) of different ages. QTf indicated $QT/RR^{1/3}$; Tpe, QT interval−Q-Tpeak interval (Q-Tpeak, the interval between the Q-point and the T-wave peak); Tpe/QT, Tpe divided by QT. Data are presented as mean±SD; p showed the probability value of univariate regression analysis between age and each ECG repolarization parameter in male or female groups, respectively.

age-dependent increases in QTc and Tpe were previously reported in healthy Japanese volunteers.[10] Because G643S SNP was associated with greater TDR-related ECG parameters (Figs 3,4), the arrhythmogenic effects of G643S appeared to be even stronger in the elderly female subgroup.

Discussion

In the present study we demonstrated that the *KCNQ1* gene SNP, G643S, has a relatively high incidence rate (8.9%) in a Japanese community population (Shigaraki) and that the heterozygous SNP carriers showed a significantly greater Tpe and Tpe/QT ratio compared to the control group selected by matching both gender and age (Fig 3). In addition, in elderly females, the TDR-related parameters including QTf were greater than in the other 3 groups, although they did not reach statistical significance (Fig 4). Thus, a larger TDR would be a risk factor for ventricular arrhythmias in SNP-positive elderly females.

In comparison with the degree of QT prolongation, Tpe and Tpe/QT values were greater in the SNP group, suggesting the presence of an abbreviation of the QTp interval. In the functional assay using a heterologous expression system,[6] G643S polymorphism was found to be functional and caused a mild reduction in I_{Ks}-like currents that were co-expressed with MinK encoded by *KCNE1* (by ~30% in the heterozygous condition). As shown in the scheme of Fig 2, the Tpe interval was obtained as the difference between the QT and QTp intervals. Meanwhile, previous studies[8,11] suggested that the net QT interval reflects the action potential duration (APD) in the subendocardial (M) layer, and the QTp interval reflects the APD in the epicardial layer. Therefore, the Tpe interval corresponds to the differences in the APDs between the 2 layers (or TDR).[7–9]

In human myocardium, electrophysiological studies have demonstrated that both epicardial and endocardial cells have stronger net inward repolarizing currents (as a result of strong I_{Ks}) compared to M cells, which is caused primarily to relatively weak I_{Ks} in the M layer.[27] In contrast, a rapidly activating component of the delayed rectifier (I_{Kr}) is distributed homogeneously in all layers and more predominantly than I_{Ks}.[28] It has also been suggested that the transient outward current, I_{to}, is more abundant in the epicardial layer than in the 2 other layers.[29] Therefore, outward potassium conductance is most scarce in the M layer, and a very mild reduction in I_{Ks} may cause a greater APD prolongation in this layer, while the epicardial APD might remain constant or even be abbreviated because other K current systems such as I_{Ks} and I_{to} could compensate for it. Roden proposed a similar mechanism as a "repolarization reserve" that modifies the arrhythmogenesis.[30] This might partially serve to explain the reason why the extension of the Tpe interval was seen without any significant QT prolongation appearing in ECGs obtained at rest.

Supposing that compensating outward K currents were decreased by the presence of additional risks such as hypokalemia and drugs associated with QT prolongation,[9] the arrhythmia risk for the SNP carriers could increase markedly. Indeed, we reported that 6 out of 95 LQTS patients

(6.3%) had the heterozygous G643S SNP but no mutations in *KCNQ1*, *KCNH2*, *SCN5A*, and *KCNE1*? Interestingly, the probands were all female with the acquired form of LQTS (mean age of 42 years). In some of these subjects, TdP was triggered by bradycardia and hypokalemia. Other unidentified genetic and/or epigenetic factors could collaborate in unmasking the latent vulnerability to arrhythmias (prolongation of QT interval or augmentation of TDR), thereby predisposing the SNP carriers to TdP.

Study Limitations

The number of SNP carriers was relatively low. When we analyzed the age-dependent effect on the ECG parameters (Fig 5), the correlation was therefore examined in the whole population. Cases under 18 years old were not included in the present study, and therefore the characteristics and the G643S SNP effect on the ventricular repolarization in childhood and adolescence were not determined. However, because the SNP was associated with the secondary type of LQTS, this was not an essential problem.

In conclusion, in our cohort of 324 individuals, *KCNQ1* G643S SNP appeared to be associated with the greater progression of the TDR-related parameters, especially in the elderly female group. It is in our hope that other prospective studies will be conducted to clarify the secondary LQTS mechanisms and gender differences.

Acknowledgments

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The Jervell and Lange-Nielsen Syndrome
Natural History, Molecular Basis, and Clinical Outcome

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Background—Data on the Jervell and Lange-Nielsen syndrome (J-LN), the long-QT syndrome (LQTS) variant associated with deafness and caused by homozygous or compound heterozygous mutations on the *KCNQ1* or on the *KCNE1* genes encoding the I_{Ks} current, are still based largely on case reports.

Methods and Results—We analyzed data from 186 J-LN patients obtained from the literature (31%) and from individual physicians (69%). Most patients (86%) had cardiac events, and 50% were already symptomatic by age 3. Their QTc was markedly prolonged (557±65 ms). Most of the arrhythmic events (95%) were triggered by emotions or exercise. Females are at lower risk for cardiac arrest and sudden death (CA/SD) (hazard ratio, 0.54; 95% CI, 0.34 to 0.88; $P=0.01$). A QTc >550 ms and history of syncope during the first year of life are independent predictors of subsequent CA/SD. Most mutations (90.5%) are on the *KCNQ1* gene; mutations on the *KCNE1* gene are associated with a more benign course. β-Blockers have only partial efficacy; 51% of the patients had events despite therapy and 27% had CA/SD.

Conclusions—J-LN syndrome is a most severe variant of LQTS, with a very early onset and major QTc prolongation, and in which β-blockers have limited efficacy. Subgroups at relatively lower risk for CA/SD are identifiable and include females, patients with a QTc ≤550 ms, those without events in the first year of life, and those with mutations on *KCNE1*. Early therapy with implanted cardioverter/defibrillators must be considered. **(*Circulation*. 2006;113:783-790.)**

Key Words: arrhythmia ■ death, sudden ■ electrocardiography ■ heart arrest ■ long-QT syndrome

In 1957, Anton Jervell (see online-only Data Supplement) and his associate, Fred Lange-Nielsen, published the first report on a familial disorder characterized by the presence of a markedly prolonged QT interval, congenital deafness, and a high incidence of sudden cardiac death in childhood.[1] Following the reports by Romano et al[2] and Ward[3] of an almost identical familial disease differing only in respect to normal hearing and the suggestion by Fraser et al[4] of a genetic relationship between the two, the two syndromes were considered variants of one disease under the unifying name of long-QT syndrome, with the acronym LQTS.[5] As we wrote 25 years ago, "There are not many instances in medical history of a single case report so critical for the development of the subsequent knowledge on a given disease."[6]

Clinical Perspective p 790

The progressive unraveling of the molecular basis of LQTS has disclosed that whereas the autosomal dominant Romano-Ward syndrome depends on mutations affecting at least 5 genes encoding sodium and potassium channels, the autosomal recessive Jervell and Lange Nielsen syndrome (J-LN) depends on homozygous or compound heterozygous mutations on either 1 of 2 genes, *KCNQ1* and *KCNE1*.[7–9] Proteins encoded by these 2 genes coassemble to form the channel

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conducting the I_{Ks} current. *KCNQ1* is also the gene responsible for LQT1, the most common form of Romano-Ward syndrome.

Being a recessive disease, J-LN is far less common than the Romano-Ward syndrome, and the reports available are based primarily on either anecdotal observations or very small series of patients, which suggests a more severe clinical course than Romano-Ward syndrome. To achieve a more thorough and quantitative understanding of this life-threatening disorder and to foster a more rational management strategy, an international cooperative effort was initiated, and the results are presented here. The data now available on 186 J-LN patients also allow a meaningful comparison between the phenotypic aspects of J-LN and of 670 LQTS patients representing the 3 major genetic subgroups (LQT1, LQT2, LQT3), as previously reported.[10]

Methods

The study population involves 186 patients from 135 families. These families originated from several countries, including Algeria, Canada, Finland, France, Germany, India, Iran, Italy, Japan, Lebanon, Morocco, Norway, Pakistan, Portugal, former Soviet Union, Spain, Sweden, Turkey, United Kingdom, United States, and former Yugoslavia. There were 2 sources of information: the international literature (31%) and individual physicians (69%). For the latter group, which also includes cases initially reported in the literature but with significant increases in follow-up, data were collected on specifically prepared forms, as previously described.[10] Patients were considered affected by J-LN on the basis of the established diagnostic criteria for LQTS[11] and on the presence of congenital neurosensory deafness. Patients who died before diagnosis and without an ECG (n=15) were considered affected by J-LN on the basis of a clear familial and clinical history, including congenital deafness. Genotyping information is available for only a few of the literature cases, largely because several patients had died or because they relate to the premolecular era; of the 97 families reported by individual physicians, 57 (59%) were successfully genotyped, mutations were not identified in 4, and the remaining families have not yet been tested.

Whenever appropriate, the data of the J-LN patients will be compared with those of 670 genotyped and symptomatic LQTS patients[10] or those of 355 LQT1 patients from the Pavia database.[12]

Cardiac Events and Triggers

Patients were considered symptomatic if they had suffered either syncope, aborted cardiac arrest, or sudden death. We also distinguished between syncope and life-threatening events (cardiac arrest and sudden death [CA/SD]).

The analysis of the triggers for cardiac events focused on 3 main conditions: exercise, emotion, and rest or sleep without arousal, as previously described.[10] We also considered the occurrence of cardiac events under "other conditions," which include all the situations different from the first 3. This reflects the fact that these other conditions are well described and often associated with cardiac events and also that the extremely young age of many patients makes the appropriate definition of intense physical exercise or of strong emotion more difficult. To avoid selection bias, whenever multiple triggers were operant in a single patient, they were all included in the analysis.[10]

Response to Therapy

Data on therapy were available for 172 patients (92%). This number also includes 36 patients who received no treatment (primarily those who died before diagnosis) and 12 patients who received therapy different from β-blockers. β-Blockers represent the most common treatment modality, involving 124 patients (91% of those being treated). The response to β-blockers was evaluated in 92 patients for whom precise information was available about both outcome and dosage. Our interest was in ascertaining whether actual treatment with β-blockers was effective; accordingly, and, as done previously,[10] 11 patients were not included in this analysis because either the dosage of β-blockers was <1.0 mg/kg per day propranolol (0.5 mg/kg per day for Japanese patients) or an equivalent dose of other β-blockers or because the therapy had been definitely discontinued. At this point the analysis was based on the intention-to-treat principle, and events occurring in relation to accidental or brief suspension of treatment were always included.

Characteristics of 186 Subjects With J-LN Syndrome

Families/probands, n	135
Female gender, n (%)	96 (52)
Family history of J-LN, n (%)	38 (28)
Consanguinity, %	35–41*
History of cardiac events, n (%)	160 (86)
Median age at first event, y (IQR)	2 (1–5)
LQTS-related death, n (%)	50 (27)
Median age at death, y (IQR)	8.5 (3.6–14)
Asymptomatic patients, n (%)	26 (14)
Age ≥15 y, n (%)	10 (5)
Median time of follow-up, y (IQR)*	13 (6–23)

*35% for all families and 41% for genotyped families only.

Statistical Analysis

Univariate analyses were performed by unpaired *t* test, ANOVA, and cross-tabulations, as appropriate. For continuous variables, data are presented as mean±1 SD or as median and interquartile range (IQR) whenever the distribution was skewed. Event-free survival was described with the use of the Kaplan-Meier life-table method. Time to first event (with birth used as time of origin) was determined by gender for any events (syncope, CA/SD, whichever occurred first) and for life-threatening events. The prognostic roles of QTc, history of syncope within the first year of life, genotype, and gender were evaluated by a Cox model, and hazard ratios (HRs) were reported with their 95% CIs. Probability values <0.05 were considered statistically significant. SPSS software (version 11.5) was used for computation.

Results

Patient Population

The clinical characteristics of the 186 J-LN patients are summarized in the Table. In 38 of the 135 families (28%), 1 or more affected individuals were identified in addition to the proband. Consanguinity was reported in 35% of these families and in 41% of the successfully genotyped families. Females (52%) and males are equally represented. Life-threatening events (CA/SD) occurred in 72 patients (39%), and among these there were 50 sudden deaths (27%): the median age at death was 8.5 years (IQR, 3.6 to 14).

The overwhelming majority of the patients (n=160; 86%) are symptomatic: among the few patients still asymptomatic (n=26), only 10 are aged ≥15 years. Thus, only 5% of this entire population approaches adulthood without having suffered cardiac events.

When patients reported by the literature were compared with those reported by individual physicians, few differences emerged. The former group showed a more prolonged QTc (592±58 versus 543±63 ms; $P<0.001$), but the prevalence of symptomatic patients and the mortality (CA/SD) rate was

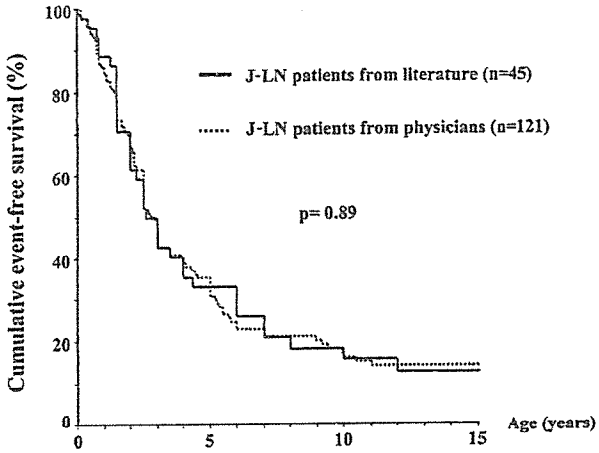

Figure 1. Kaplan-Meier curves of event-free survival according to the source of information.

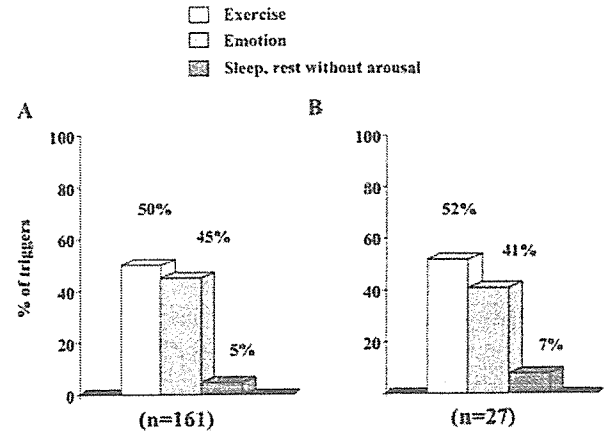

Figure 3. Conditions (triggers) associated with the occurrence of all cardiac events (A) and of life-threatening cardiac events only in the J-LN patients (B). Numbers in parentheses indicate numbers of triggers.

not significantly different between the 2 sources, with a median event-free survival time of 31 and 35 months (P=0.89) (Figure 1).

Time to First Event

The availability for 140 patients (88% of those with symptoms) of their age at first event has allowed the presentation of survival curves (interval from birth to first cardiac event). Among the J-LN patients, 15% becomes symptomatic within the first 12 months of life; the median time (50%) of survival free from cardiac events is 33 months, and by age 18 years 90% of them have had a first cardiac event (Figure 2A, 2B). This is in striking contrast to the Romano-Ward patients and especially the LQT2 and LQT3 subtypes, which manifest their symptoms much later in life (Figure 2C); of note, all of these Romano-Ward patients were symptomatic.[10] Figure 2D shows how this difference in severity becomes even more striking when compared with a large group of unselected LQT1 patients referred to a single center.[12]

Triggers for Cardiac Events

Figure 3 shows the prevalence of the various triggers for all cardiac events (Figure 3A) and for life-threatening cardiac events (Figure 3B). Exercise and emotions, which are both conditions associated with increased sympathetic activity, are equally important, and they account for 95% of cardiac events. Very few events (5%) were associated with rest or sleep. Among specific activities, swimming is notable because it is associated with events in 16% of all patients with known triggers.

When the analysis is limited to life-threatening events, it appears that 93% of them occur during exercise and emotions and only 7% during sleep/rest. In addition to those patients

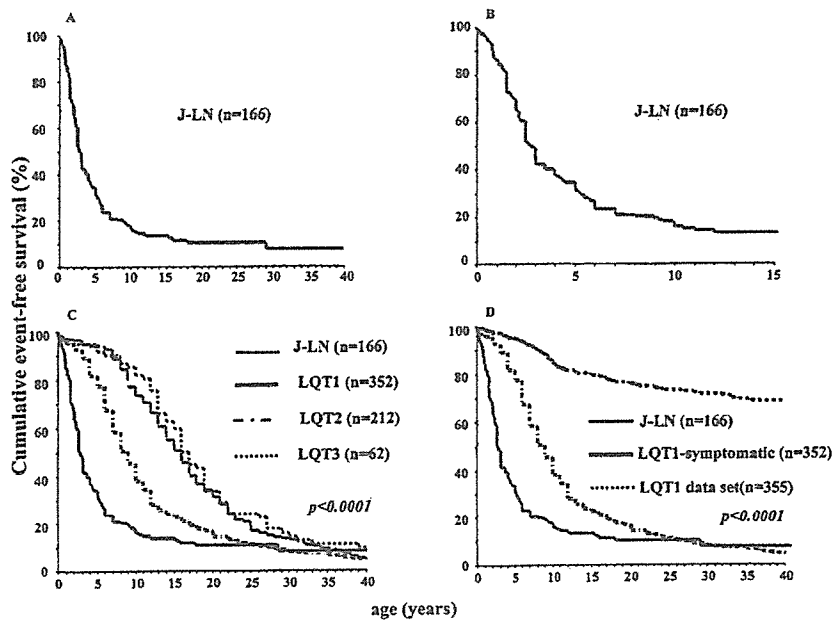

Figure 2. Kaplan-Meier curves of event-free survival. A, All J-LN patients; B, all J-LN patients with a magnified view of the events occurring in the first 15 years of life; C, J-LN patients vs Romano-Ward symptomatic patients of known genotype[10]; D, J-LN patients vs LQT1 symptomatic patients[10] and an unselected LQT1 population.[12]

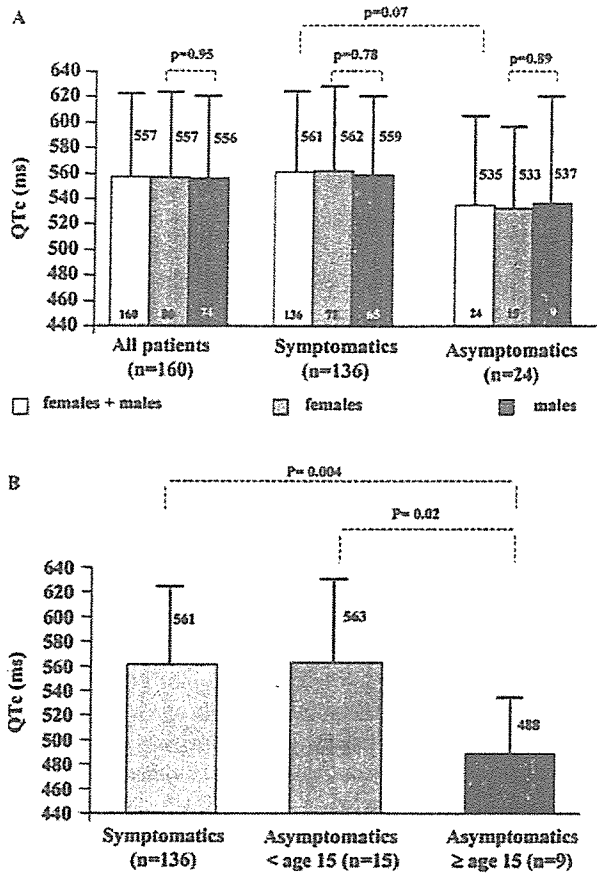

Figure 4. QTc in the J-LN population according to the presence of symptoms and gender (A) and according to the presence of symptoms and age of the patients (B).

who had life-threatening events with the 3 main triggers, 15 (37%) suffered a CA/SD associated with "other conditions" (fever, pregnancy, anesthesia, normal daily activities, sepsis with low K^+, diarrhea).

QT Interval Duration

QTc measurements were available for 160 patients (86%). The QTc of the J-LN patients was markedly prolonged (557±65 ms), with no difference between females and males (557±66 and 556±65 ms, respectively) (Figure 4A). Surprisingly, the QTc of symptomatic patients was not significantly longer than that of patients without symptoms (561±64 versus 535±71 ms; P=0.07). However, when the truly asymptomatic subjects (aged ≥15 years: n=10 aged 15 to 83 years) were compared with the younger asymptomatic subjects (n=16 aged 1 to 13 years), who still have a high probability of becoming symptomatic, an important finding emerged. The QTc of the very young asymptomatic subjects (563±70 ms) is similar to that of the symptomatic subjects (561±64 ms), which is significantly longer than that of the patients truly likely to remain asymptomatic (488±47 ms; P=0.004) (Figure 4B).

Figure 5 shows that the QTc is longer among J-LN symptomatic patients with CA/SD than among those with syncope (585±64 versus 545±58 ms: P<0.001) and that the

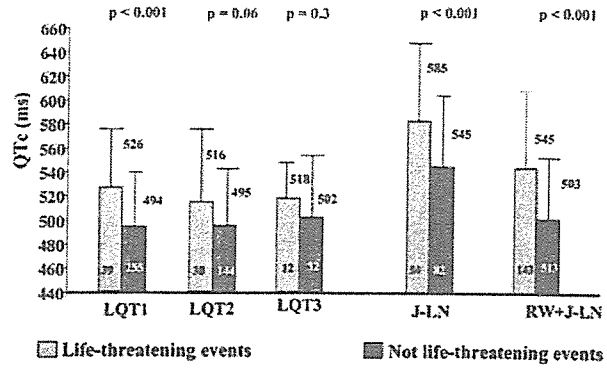

Figure 5. QTc in symptomatic J-LN and Romano-Ward (RW) patients of known genotype, according to the severity of cardiac events (syncope only vs life-threatening episodes).

same pattern (526±49 versus 494±45 ms; P<0.001) is present among symptomatic LQT1 patients.[10] By contrast, no significant difference was present among LQT2 and LQT3 patients despite a trend in the same direction. When all LQTS patients (Romano-Ward and J-LN) are analyzed together, those with CA/SD have a longer QTc (545±65 vs 503±51; P<0.001).

Role of Gender

Females are at lower risk for life-threatening events (HR, 0.54; 95% CI, 0.34 to 0.88; P=0.01). Although a pattern (P=0.05) is already present when all cardiac events are considered (Figure 6A), this difference becomes more evident (P=0.01) when syncope is excluded and the analysis is limited to life-threatening events (CA/SD; Figure 6B) or to sudden death only (P=0.02; Figure 6C). These cumulative survival estimates do not take into account the potential confounding role of β-blocker therapy used in 63% and 70% of females and males, respectively.

Genetics

The genotype was known for 63 (47%) of the 135 families. In most families (57 of 63; 90.5%) the mutations were on the *KCNQ1* gene, whereas in 6 (9.5%) they were on *KCNE1*. Among the genotyped probands, 33% were compound heterozygous. They and the homozygous subjects were not different for all the variables examined: QTc duration, prevalence of symptoms, gender, age at first episode, type of triggers, lethal episodes regardless of therapy, and response to β-blockers. These results also apply when the analysis is limited to the *KCNQ1* subgroup.

In the *KCNQ1* group, complex mutations (insertions/deletions, splice variants, truncations) in at least 1 allele were found in 74% of probands. There was no difference in QTc duration, symptoms, and life-threatening events between patients with at least 1 complex mutation and those with missense mutations.

Among the 77 symptomatic patients of known genotype, 73 (95%) have mutations on *KCNQ1*, and 4 (5%) have mutations on *KCNE1*. This distribution is significantly different (P=0.001) than that seen in the 6 truly asymptomatic successfully genotyped patients (aged ≥15 years), among

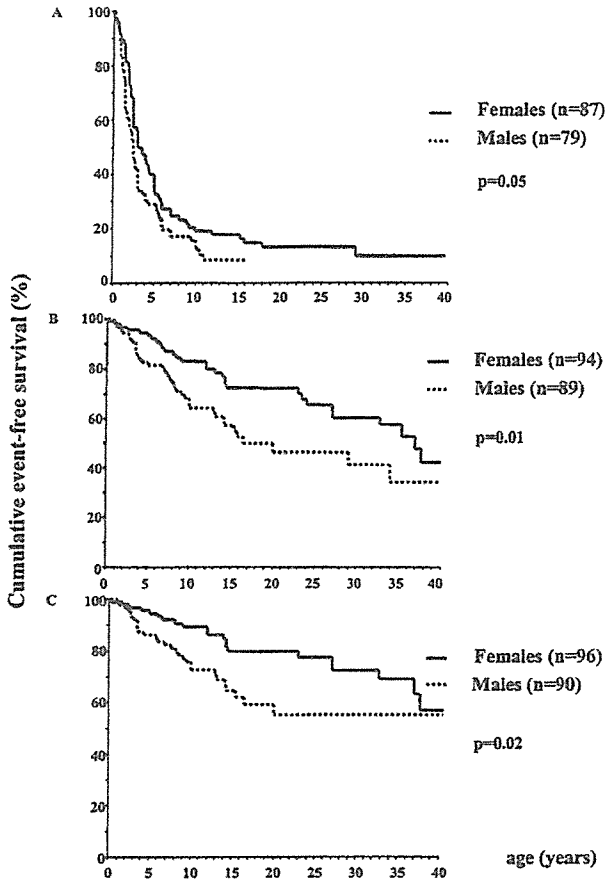

Figure 6. Kaplan-Meier curves of event-free survival and survival in the J-LN patients, according to gender. A, Any first event; B, life-threatening events (CA/SD); C, sudden death only. Numbers in parenthesis are number of patients for whom information on the time of the event is available.

whom only 2 (33%) have mutations on *KCNQ1*, whereas 4 (67%) have mutations on *KCNE1*. In addition, a significantly longer QT interval was observed among patients with *KCNQ1* mutations than among *KCNE1* mutation carriers (556±55 versus 517±72; $P=0.03$). As shown in Figure 7, the cumulative probability of a first cardiac event is significantly lower for J-LN patients with *KCNE1* mutations than for those carrying *KCNQ1* mutations ($P=0.0003$). Even after we accounted for QTc and gender, *KCNE1* mutations remain associated with a lower risk for arrhythmic events compared with *KCNQ1* mutations, as shown in a Cox model by HR of 0.18 (95% CI, 0.06 to 0.50; $P=0.001$).

Prognostic Factors and Risk Stratification

After adjustment for gender, multivariate Cox regression analysis indicated that both a QTc ≤550 ms (ie, the median value of QTc in the entire J-LN population) and no history of syncope in the first year of life were significantly associated with a lower risk of a subsequent life-threatening event (HR, 0.36; 95% CI, 0.19 to 0.68; $P=0.002$; HR, 0.44; 95% CI, 0.22 to 0.87; $P=0.02$, respectively). In this multivariate analysis, gender was also an independent risk factor ($P=0.03$), with a lower risk for females than for males (HR, 0.51; 95% CI, 0.27 to 0.95).

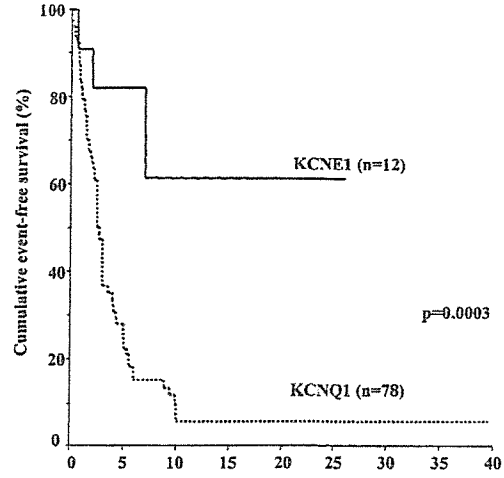

Figure 7. Kaplan-Meier curve of event-free survival in J-LN patients with mutations in *KCNQ1* or *KCNE1* genes.

To further define the risk of life-threatening events, the J-LN population was stratified according to the 4 possible combinations of QTc duration and history of early syncope. Figure 8 shows that the combined presence of a QTc >550 ms and of syncope during the first year of life is associated with a markedly increased probability of CA/SD during follow-up ($P<0.001$) compared with the absence of both or only 1 of these risk factors.

β-Blocker Therapy

Among the 124 patients who were prescribed β-blockers, follow-up data were available for 103 (83%); however, 11 met the exclusion criteria and were considered off-therapy. Thus, 92 patients met the criteria for assessing β-blocker efficacy. Among them, 69 (75%) had experienced at least 1 cardiac event before the institution of β-blocker therapy, and 23 (25%) were asymptomatic before treatment was started. In total, 45 (49%) became or remained asymptomatic while on therapy, whereas the remaining 47 (51%) developed recurrences or experienced their first cardiac episode after initiation of therapy. There were 10 cardiac arrests and 15 sudden deaths, for a total of 27% of patients on therapy who suffered CA/SD. The median age at onset of therapy was 3.5 years (IQR, 1 to 7) (19 patients started on β-blockers soon after birth or within the first year), and the median duration of therapy was 8 years (IQR, 3 to 15). The median age at death on therapy was 8 years (IQR, 6 to 14).

Additional Therapies

In 32 of the 92 patients analyzed for the efficacy of β-blockers, 1 or more additional therapies were used, including pacemakers (n=12), implantable cardioverter/defibrillators (ICDs) (n=13), and left cardiac sympathetic denervation (LCSD) (n=16). In 18 of these 32 patients (56%), there were additional recurrences, including 7 sudden deaths. Recurrences occurred in 8 of 11 patients with pacemakers, in 9 of 16 patients with LCSD, and in 4 of 13 patients with an ICD (appropriate shocks).

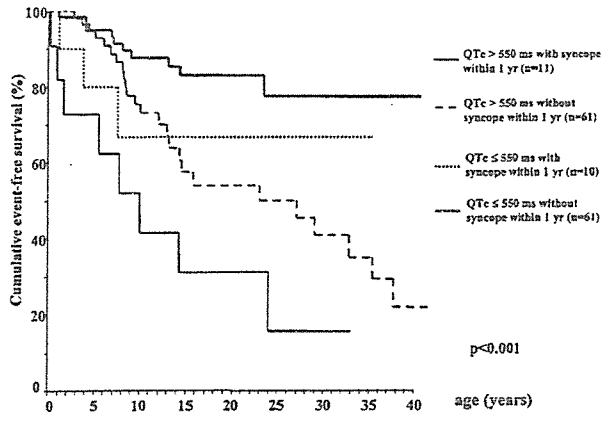

Figure 8. Kaplan-Meier curves of life-threatening (CA/SD) events: cumulative survival in J-LN patients, according to the combinations of a QTc ≤550 or >550 ms and the presence/absence of a history of syncope during the first year of life.

Discussion

This cooperative study provides information on an unprecedented number of patients affected by the J-LN syndrome and allows for the first time a meaningful assessment of the main features of this intriguing disorder. The data show specific differences in terms of clinical manifestations and response to therapy with all subsets of LQTS. Even when compared with LQT1, the LQTS variant that shares with J-LN an impairment of the I_{Ks} current, there are both similarities and important differences.

The J-LN syndrome has a special place in cardiology because it was its recognition by Anton Jervell,[1] followed by his additional reports,[13,14] which paved the way for the first reports on the same cardiac disorder without congenital deafness, the LQTS, which has been correctly described as a Rosetta stone for sudden cardiac death.[15] Indeed, the identification of several of the LQTS genes has represented a major breakthrough for cardiology and for the study of cardiac arrhythmias by providing a previously unforeseen bridge between molecular biology and clinical cardiology.

The large numbers of the present study allow us to draw conclusions concerning the natural history of J-LN, the genotype-phenotype correlation, the risk factors for life-threatening cardiac events, and the response to therapy.

Natural History

As suspected from the early cases, and with the possible exception of the very rare forms of LQTS with congenital A-V block[16] and with syndactyly,[17] the J-LN is the most severe of the major variants of LQTS. This is exemplified by the fact that almost 90% of the patients become symptomatic and that sudden death exceeds 25% despite medical therapy. Furthermore, the J-LN patients begin to suffer cardiac events very early in life. During the first year of life 15% already had an event, by age 3 years 50% have had an event, and by age 18 years a staggering 90% had symptoms.

The uniquely early onset of symptoms is clearly illustrated by the comparisons with LQT1, LQT2, and LQT3 patients selected for having a severe form of LQTS because they were all symptomatic. This difference is further amplified when one compares all the J-LN patients not just with symptomatic LQT1 patients but especially with an unselected large group of LQT1 patients that, because of low penetrance,[18] includes a significant proportion of silent mutation carriers and of asymptomatic individuals.

Even though these data include a number of patients identified many years ago when diagnosis was likely to be made only in the most severe cases, and indeed the QTc of patients reported from the literature is more prolonged, the difference in mortality is relatively modest compared with that of patients with direct information from the responsible physicians.

The conditions that trigger the cardiac events are, overall, very similar to those described for LQT1,[10] as expected for mutations affecting the I_{Ks} current. Most of these conditions (95%) involve sympathetic activation and are represented by exercise and emotions, and only 5% of the events occur at rest or during sleep. This observation confirms the specific relation between genotype and triggers for cardiac events that we postulated in 1995[19] and confirmed in 2001.[10]

QTc duration is also a major risk factor for J-LN patients, and it is much longer than in any other LQTS genetic group. This likely reflects the "double-hit," the presence of 2 mutations, with the attendant greater loss in repolarizing current. QTc duration offers interesting insights for the possible prediction of those young patients more likely to remain asymptomatic throughout life. Indeed, analysis of the QTc of the still asymptomatic patients becomes informative when their age is taken into account. As Figure 2A shows that only 2% of patients become symptomatic after age 15 years, J-LN individuals without symptoms by this age can represent the group of "true asymptomatic" patients. The mean QTc of these individuals (488 ms) is significantly shorter than that of all the symptomatic patients (561 ms) and of patients who are still asymptomatic but aged <15 years (563 ms) who actually have a high probability of becoming symptomatic. The fact that QTc is not different between males and females, which is at clear variance with most LQTS patients, is surprising only at first glance because it is probably explained by the very young age of most patients. Even among normal individuals, the longer QTc associated with the female gender is absent at birth.[20]

The gender issue is more important in relation to the severity of the arrhythmic events. Indeed, the probability of a J-LN patient developing CA/SD is markedly higher for males. Besides gender, multivariate analyses identifies other 2 independent risk factors for the life-threatening events, namely, a QTc >550 ms and occurrence of syncope during the first year of life.

Molecular Basis

As expected on the basis of the distribution of genotypes in LQTS,[20] most of the J-LN mutations are on the *KCNQ1* gene. Although this distribution is replicated among the symptomatic patients, the pattern among the small group of genotyped asymptomatic patients is profoundly different because most of them are *KCNE1* mutation carriers. Furthermore, a multivariate analysis shows that patients with *KCNQ1* mutations have an almost 6-fold greater risk of arrhythmic events. Thus,

within the J-LN population it is possible to recognize a genetic subgroup at lower risk, namely, the patients with *KCNE1* mutations. It follows that to genotype all J-LN patients should be regarded as correct management and not as a research objective.

A puzzling problem continues to be the reason why LQT1 patients are much more symptomatic and at risk for lethal events than the parents of the J-LN patients, despite the fact that they all are heterozygous for the same gene. The most obvious explanation suggests that J-LN mutations are "milder" and therefore, in the heterozygous form, do little harm. Some data support this concept. Indeed, most of the LQT1 genetic variants are missense mutations[21,22] exerting a dominant negative effect because they can coassemble with normal subunits and interfere with channel function. On the other hand, the present data demonstrate that most (74%) of J-LN mutations on *KCNQ1* are complex mutations, likely to interfere with subunit assembly. This confirms, in a much larger and more heterogeneous group of J-LN families, observations previously published in small series.[23–25] However, exceptions exist, including both dominant negative J-LN mutations[23] and autosomal recessive Romano-Ward mutations with mild electrophysiological alterations.[26,27] These still imperfect genotype-phenotype correlations point to the likely existence of additional factors, such as modifier genes,[28] that alter the clinical severity resulting from specific mutations.

Consanguinity was reported in 41% of the J-LN probands' parents, apparently in contrast with the finding that 67% of the successfully genotyped probands are homozygous. This might reflect a bias in reporting consanguinity but could also suggest that mild mutations spread more easily in close communities, thus increasing the chances to produce homozygous carriers.

Implications for Therapy

LQT1 patients are at high risk during sympathetic activation and are very well protected by β-blockers, as shown by the incidence of only 1.6% and 1.1% of CA/SD in 2 large studies performed in referral centers[29,30] and also among children.[31] This is in striking contrast to the 27% of J-LN patients who suffered CA/SD despite being treated with β-blockers. In addition, an impressive 51% of patients remained or became symptomatic while on therapy. The figures of this clear failure of β-blockers to provide adequate protection from arrhythmic events are even worse than those previously reported for LQT3 patients, namely, 17% and 14% for combined CA/SD.[10,29]

These grim figures are compounded by the fact that a high incidence of recurrences is recorded even when β-blockers are associated with additional therapies such as pacing or LCSD. The limited efficacy of LCSD is at variance with the encouraging results obtained even in the difficult-to-manage LQT3 group.[32]

The very limited efficacy of β-blockers for J-LN patients is alarming and indicates the need for more aggressive therapy in at least half of them. Risk stratification including genotype, age at diagnosis, symptoms at presentation, degree of QT-interval prolongation, and gender may help identify patients at higher risk of life-threatening events. Neither LCSD nor antibradycardia pacing was protective against life-threatening events in patients known to be at increased risk, which suggests that ICD implantation may be required. Young J-LN children are at particularly high risk for cardiac events, as 50% of them experienced events by 3 years of age. These data suggest that J-LN children with high-risk characteristics should be considered for defibrillator implantation. Young J-LN children and infants without identified high-risk characteristics should have an external defibrillator available in addition to medications. Improvements in defibrillator technology that allow implantation of smaller epicardial systems are needed. LCSD, given its efficacy in LQTS patients with storms of shocks by the ICD,[32] has a place in all J-LN patients, with the goal of minimizing the probability of an ICD shock, which, especially in young children, has a high potential to trigger storms of shocks as a result of pain, fright, and further release of catecholamines.

The present study offers the first possibility of selecting for these young children more or less aggressive therapies on the basis of data-driven risk stratification. Indeed, because a J-LN child with a QTc <550 ms and without syncope in the first year of life has ≈90% probability of not suffering CA/SD events before age 8 to 10 years, in this group it would be possible to wait a few years before the decision is made to implant an ICD. The presence of female gender and/or of *KCNE1* mutations can also usefully contribute to individually tailored management of this life-threatening disease.

For J-LN patients, aggressive efforts to risk-stratify and to provide tiered therapy based on risk are absolutely essential.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The Jervell and Lange-Nielsen syndrome (J-LN) is the long-QT syndrome variant associated with congenital deafness. It is caused by homozygous or compound heterozygous mutations on the *KCNQ1* or the *KCNE1* genes encoding the I_{Ks} current. Fifty years after its first description, the information on J-LN is still based largely on anecdotal case reports. We have gathered accurate clinical and genetic data on 186 J-LN patients. Most patients (86%) had cardiac events, and 50% were symptomatic by age 3. Their QTc was markedly prolonged (557±65 ms), and most arrhythmic events (95%) were triggered by emotional or physical stress. β-Blockers were modestly protective: 51% of patients had recurrences under therapy, and 27% had cardiac arrest or sudden death (CA/SD). The severity of the clinical manifestations, early occurrence of life-threatening arrhythmias, and poor response to medical therapy indicate the need to resort to use of an implantable cardioverter/defibrillator (ICD). Given the complications associated with ICD therapy in young children, we focused our analysis on the identification of subgroups of patients at lower risk for whom ICD implantation could be delayed. We found that 4 factors (QTc <550 ms; no history of syncope during the first year of life; female gender; mutations on the *KCNE1* gene) were associated with a significantly lower risk for CA/SD. This novel information allows, for the first-time, physicians managing J-LN patients to make therapeutic choices based on a data-driven individualized risk stratification.

Angiotensin II Potentiates the Slow Component of Delayed Rectifier K^+ Current via the AT_1 Receptor in Guinea Pig Atrial Myocytes

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Background—Angiotensin II (Ang II) has diverse actions on cardiac electrical activity. Little information is available, however, regarding immediate electrophysiological effects of Ang II on cardiac repolarization.

Methods and Results—The present study investigated the immediate effects of Ang II on the slow component of delayed rectifier K^+ current (I_{Ks}) and action potentials in guinea pig atrial myocytes using the whole-cell patch-clamp technique. Bath application of Ang II increased the amplitude of I_{Ks} (EC_{50}, 6.16 nmol/L) concentration dependently. The stable analogue Sar^1–Ang II was also effective at increasing I_{Ks}. The voltage dependence of I_{Ks} activation and the kinetics of deactivation were not significantly affected by these agonists. The enhancement of I_{Ks} was blocked by the Ang II type 1 (AT_1) receptor antagonist valsartan (1 μmol/L) and was markedly attenuated by inclusion of GDPβS (2 mmol/L) in the pipette, indicating an involvement of G protein–coupled AT_1 receptor. The stimulatory effect was also significantly reduced by the phospholipase C inhibitor compound 48/80 (100 μmol/L) and the protein kinase C inhibitors bisindolylmaleimide I (200 nmol/L) and H-7 (10 μmol/L), suggesting that AT_1 receptor acts through phospholipase C–protein kinase C signaling cascade to potentiate I_{Ks}. As expected from its stimulatory action on I_{Ks}, Sar^1–Ang II markedly shortened the action potential duration, which could be reversed by valsartan.

Conclusions—The potentiation of I_{Ks} via AT_1 stimulation in atrial myocytes, accompanied by a shortening of the action potential duration, suggests a potential mechanism by which elevated levels of Ang II may promote atrial fibrillation in heart failure and warrants further investigation. (***Circulation.* 2006;113:1278-1286.**)

Key Words: action potentials ■ angiotensin ■ atrium ■ ion channels ■ receptors

The renin-angiotensin system (RAS) plays a fundamental role in maintaining cardiovascular homeostasis, and disorders of the RAS are closely related to the development of hypertension, heart failure, atherosclerosis, cardiac hypertrophy, and myocardial and vascular remodeling.[1–5] An octapeptide angiotensin II (Ang II) is the principal effector of the RAS and produces its potent and diverse biological actions by interacting with specific membrane receptors, namely Ang II type 1 (AT_1) and type 2 (AT_2) receptors. The AT_1 receptors are widely distributed in a variety of cell and tissue types and mediate most of the known actions of Ang II, whereas the AT_2 receptor is expressed mainly in the embryonic and neonatal states but is upregulated in adult tissues under some pathological conditions.[6] The various biological actions of Ang II via AT_1 receptor can be divided into the short-term effects that occur within minutes and the long-term effects that take place within hours or even later; vascular contraction constitutes the short-term events, whereas the long-term effects include a transcriptional response that leads to morphological and functional alterations of cardiovascular systems such as cardiac hypertrophy, fibrosis, and remodeling.[7]

Clinical Perspective p 1286

There is increasing evidence that the RAS is also associated with the occurrence of atrial and ventricular arrhythmias in experimental animals,[8,9] and recent clinical studies have proved that blockade of the RAS with ACE inhibitors or AT_1 antagonists is effective for the treatment of atrial fibrillation (AF).[10,11] The shortening of action potential duration (APD) and effective refractory period can be regarded as one of the main factors responsible for the occurrence of reentry-based tachyarrhythmias such as AF. Little information is available, however, regarding the effect of Ang II on repolarizing K^+ currents and resultant changes in APD in cardiac myocytes.

It has been demonstrated in various mammalian species, including humans, that the delayed rectifier K^+ current (I_K) consists of rapidly and slowly activating components (I_{Kr} and I_{Ks}, respectively), which are the major repolarizing outward

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