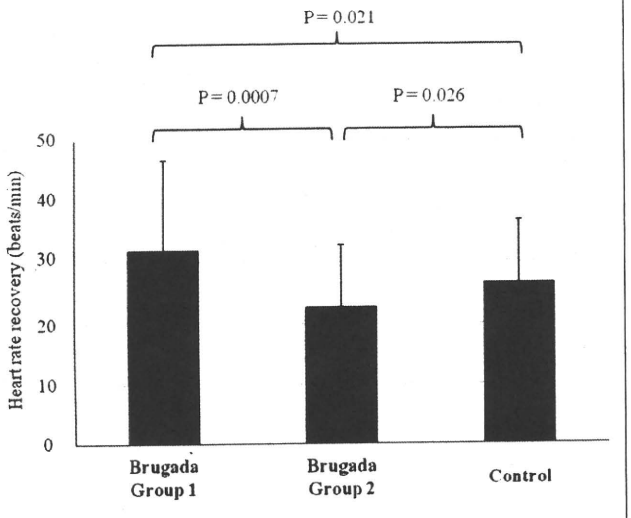

Figure 3 **Comparison of HRR After Exercise Cessation**

Comparison of heart rate recovery (HRR) 1 min after exercise cessation among Brugada syndrome patients of groups 1 and 2 and control subjects. The HRR in group 1 patients was significantly larger than that in group 2 (32 ± 15 beats/min vs. 23 ± 10 beats/min, p = 0.0007) and in control subjects (32 ± 15 beats/min vs. 26 ± 10 beats/min, p = 0.021). The differences of HRR between group 2 patients and control subjects were also significant (23 ± 10 beats/min vs. 26 ± 10 beats/min, p = 0.026).

0.068) (Fig. 4D). The results of Cox regression analysis are shown in Table 3. In univariate analysis, indexes predictive of cardiac events were previous episodes of VF (p = 0.003), ST-segment augmentation at early recovery (group 1; p = 0.005), and presence of *SCN5A* mutation (p = 0.037). In multivariate Cox regression analysis, previous episodes of VF and ST-segment augmentation at early recovery were significant and independent predictors of subsequent cardiac events (p = 0.005 and p = 0.007, respectively).

The incidence of cardiac events during follow-up in the subgroups according to symptoms before exercise testing is shown in Table 4. In the subgroup of 35 BrS patients with syncope alone, group 1 had a significantly higher cardiac event rate than group 2 (log-rank, 6 of 12 [50%] vs. 3 of 23 [13%], p = 0.016). Of note, among 36 asymptomatic patients, only 3 patients (9%) in group 1 experienced cardiac events. The log-rank test also demonstrated higher cardiac event risk in group 1 compared with group 2 (3 of 15 [20%] vs. 0 of 21 [0%], p = 0.039).

Discussion

The major findings of the present study were the following: 1) 37% of BrS patients showed ST-segment augmentation at early recovery during exercise testing; 2) ST-segment augmentation at early recovery was specific in BrS patients, and was significantly associated with a higher cardiac event rate, notably for patients with previous episode of syncope or for asymptomatic patients; and 3) BrS patients with ST-segment augmentation at early recovery showed significantly larger HRR. This is the first systematic report on the relationship between ST-segment augmentation during recovery from exercise and prognosis for BrS patients.

Augmentation of ST-segment elevation and possible mechanism. It is well known that autonomic function influences an extent of ST-segment elevation in BrS (8). The ST-segment elevation is mitigated by administration of β-adrenergic agonists and is enhanced by parasympathetic agonists such as acetylcholine in experimental and clinical investigations (5,14–16). Parasympathetic reactivation is thought to occur at early recovery after treadmill exercise testing, especially in the first minute after cessation of exercise (10,17). In the present study, we measured the ST-segment amplitude as a repolarization parameter rather than a depolarization parameter, and evaluated HRR to investigate the correlation between ST-segment augmentation and parasympathetic activity (9,18). The BrS patients who had ST-segment augmentation had significantly larger HRR compared with patients who did not, suggesting that the ST-segment augmentation was closely related to higher parasympathetic activity. However, it is still unclear whether ST-segment augmentation observed in the 34 BrS patients was simply due to more increased parasympathetic activity or to more increased susceptibility (hypersensitivity) to the parasympathetic reactivation.

Conversely, the *SCN5A* mutation was more frequently identified in group 1. Scornik et al. (19) reported that *SCN5A* mutation can accentuate parasympathetic activity toward the heart directly. It was also reported that specific mutations in the *SCN5A* gene may lead to augmentation of J-point amplitude or ST-segment amplitude during beta-adrenergic stimulation (20,21). Veldkamp et al. (20) demonstrated that a specific *SCN5A* mutation, 1795insD, augments slow inactivation, and delays recovery of sodium channel availability, thus reducing the sodium current and resulting in augmented peak J-point amplitude at rapid heart rate. Increased body temperature induced by exercise can be a risk of life-threatening arrhythmias in patients with BrS (22). A specific *SCN5A* missense mutation, T1620M, was reported to cause a faster decay of the sodium channel but slower recovery from inactivation, resulting in increased ST-segment elevation in precordial leads at higher temperatures during exercise. Although Amin et al. (13) reported that exercise induced augmentation of peak J-point amplitude, a depolarization parameter or at least combined parameter of both depolarization and repolarization, in all subjects tested, the incidence of increase in the peak J-point amplitude at peak exercise was lower (37%) in our Brugada patients. This is probably in part because only 9 (10%) of our 93 BrS patients had the *SCN5A* mutation. We could not identify significant differences in HRR, QRS duration, peak J-point amplitude (lead V_2), and ST-segment amplitude (leads V_1, V_2, V_3) at peak exercise between patients with and without *SCN5A* mutation (not shown), and that may be also due to the small number of BrS patients with *SCN5A* mutation.

Risk stratification in BrS. Implantation of an ICD is a first line of therapy for secondary prevention in patients with BrS who exhibited previous history of VF. The American College

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Table 2 Clinical, Laboratory, Electrocardiographic, and Electrophysiologic Characteristics and Long-Term Follow-Up of Groups 1 and 2 Brugada Syndrome Patients

Characteristic	Group 1 (n = 34)	Group 2 (n = 59)	p Value
Clinical characteristics			
Age at exercise testing, yrs	42 ± 11	48 ± 15	NS
Men	34 (100%)	57 (97%)	NS
Family history of SCD at age <45 yrs or Brugada syndrome	7 (21%)	16 (27%)	NS
Documented AF	7 (21%)	12 (20%)	NS
Documented VF before exercise testing	7 (21%)	15 (25%)	NS
Syncope alone before exercise testing	12 (35%)	23 (39%)	NS
Asymptomatic before exercise testing	15 (44%)	21 (36%)	NS
Age at first cardiac event, yrs	42 ± 13	45 ± 15	NS
ICD implantation	25 (74%)	38 (64%)	NS
Laboratory characteristics			
SCN5A mutation	6 (17%)	3 (5%)	0.048
Electrocardiographic characteristics			
RR, ms	951 ± 170	953 ± 140	NS
PR, ms	184 ± 28	175 ± 31	NS
QRS, ms	98 ± 14	98 ± 17	NS
QTc, ms	418 ± 46	415 ± 43	NS
ST-segment amplitude (mV) at baseline			
V_1	0.14 ± 0.09	0.16 ± 0.12	NS
V_2	0.41 ± 0.22	0.38 ± 0.26	NS
V_3	0.22 ± 0.13	0.19 ± 0.14	NS
Spontaneous coved-type ST-segment elevation in right precordial leads	30 (88%)	43 (73%)	NS
Signal-averaged electrocardiogram			
TfQRS, ms	122 ± 15	118 ± 17	NS
Late potential	28/34 (82%)	30/57 (53%)	0.004
Premature ventricular complexes during exercise	8 (24%)	11 (19%)	NS
Premature ventricular complexes at recovery	10 (29%)	9 (15%)	NS
Electrophysiologic characteristics			
AH interval, ms	107 ± 24	98 ± 27	NS
HV interval, ms	45 ± 8	44 ± 11	NS
Induction of VF	26/31 (84%)	33/47 (70%)	NS
Follow-up			
Cardiac events	15 (44%)	10 (17%)	0.004
Follow-up period, months	74.1 ± 42.2	76.5 ± 36.4	NS

AF = atrial fibrillation; ICD = implantable cardioverter-defibrillator; SCD = sudden cardiac death; TfQRS = total filtered QRS duration; VF = ventricular fibrillation; other abbreviations as in Table 1.

of Cardiology/American Heart Association/Heart Rhythm Society guidelines refer to BrS patients who have had syncope as having Class IIa indication for ICD therapy (23). However, there is still much room for argument with respect to treatments for patients who have had only syncope, and for asymptomatic patients (24–28). Although inducibility of VF during EPS (25,26), family history of SCD (24), spontaneous type 1 ECG (25,27), and late potential (28) have been proposed as predictors of cardiac events, the availability of these indexes remains controversial (7,29).

In the present study, a previous episode of VF (or aborted cardiac arrest) was the strongest predictor of subsequent cardiac events, as in previous studies (7,30,31). Moreover, ST-segment augmentation at early recovery during exercise testing was a significant and independent predictor of subsequent cardiac events in the present study. The results suggested that parasympathetic activity plays an important role in both ST-segment augmentation and subsequent cardiac events. As previously noted, it remains unclear that the cause of ST-segment augmentation in our 34 patients was a result of more increased parasympathetic activity or of more increased susceptibility of the patients to the increased parasympathetic reactivation.

Study limitations. First, BrS patients were confined to those who were hospitalized in our hospital for close investigation. That indicates these patients can be biased toward relatively high risk. Second, the present study is based on data from a small population of 93 patients; hence, it was not sufficient to evaluate the prognosis, and there also was a small number of events. Although we adopted a step-wise approach, the limited number of events can lessen the precision of the consequences for multivariate Cox regression analysis.

Conclusions

The presence of *SCN5A* mutation was a significant predictor of subsequent cardiac events by univariate Cox regression analysis. However, multivariate Cox regression analysis showed it was not a significant predictor of prognosis.

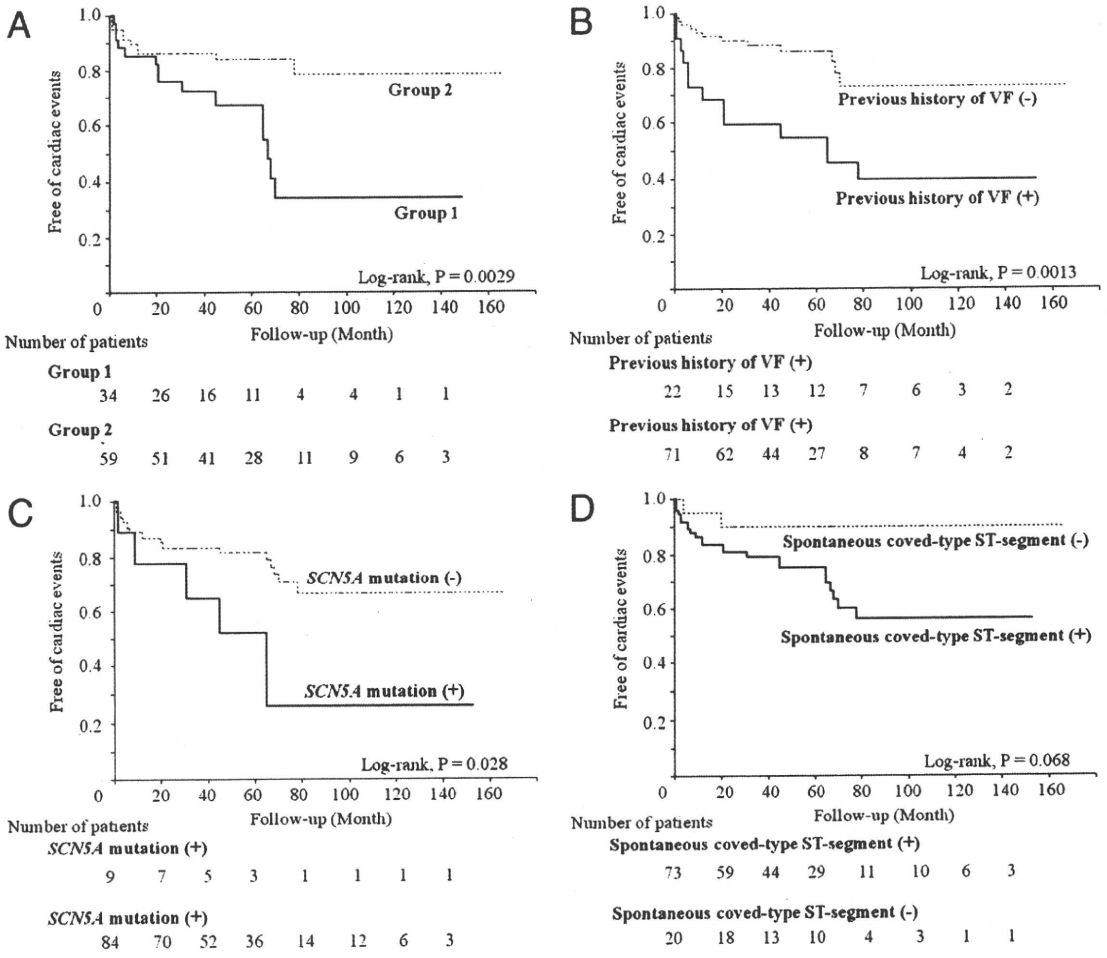

Figure 4 **Kaplan-Meier Analysis of Cardiac Events During Follow-Up**

Kaplan-Meier analysis of **(A)** cardiac events during follow-up, depending on patterns in response to ST-segment elevation during exercise test (groups 1 and 2), **(B)** incidence of previous episode of ventricular fibrillation (VF), **(C)** *SCN5A* mutation, and **(D)** spontaneous coved-type ST-segment elevation. Group 1 Brugada patients had a significantly higher cardiac event rate than did group 2 Brugada patients (log-rank, p = 0.0029). Brugada patients with previous episodes of VF or with *SCN5A* mutation had significantly greater values for occurrence of subsequent cardiac events than did patients without VF episodes or *SCN5A* mutation (p = 0.0013, p = 0.028, respectively), whereas spontaneous coved-type ST-segment elevation in Brugada patients did not predict cardiac events compared with patients not having such ST-segment elevation (p = 0.068).

Further study with a larger number of BrS patients will be required to evaluate the significance of the index as a predictor of subsequent cardiac events.

As for BrS patients with only syncope, subsequent cardiac events occurred in 50% (6 of 12) patients who exhibited ST-segment augmentation at early recovery. Asymptomatic

Table 3 **Predictive Capabilities of Cardiac Events**

		Univariate Analysis		Multivariate Analysis	
	Positive, n (%)	HR (95% CI)	p Value	HR (95% CI)	p Value
Previous episodes of VF	22 (24%)	3.40 (1.54–7.53)	0.003	3.25 (1.43–7.37)	0.005
Augmentation of ST-segment elevation at early recovery phase	34 (37%)	3.17 (1.42–7.09)	0.005	3.17 (1.37–7.33)	0.007
SCN5A mutation	9 (10%)	2.86 (1.07–7.66)	0.037		
Spontaneous coved-type ST-segment	72 (77%)	3.51 (0.83–14.9)	0.089		
Late potential	58/91 (64%)	2.25 (0.84–5.99)	0.11		
VF inducible in EPS	59/78 (76%)	0.73 (0.30–1.75)	0.48		
Family history of SCD or BrS	23 (25%)	1.19 (0.47–3.02)	0.72		

BrS = Brugada syndrome; CI = confidence interval; EPS = electrophysiologic study; HR = hazard ratio; other abbreviations as in Table 2.

Table 4 Incidence of Cardiac Events According to Symptoms Before Exercise Testing

Type	n	Treadmill Exercise Test	n	VF Occurrence	p Value (vs. Group 1)
Documented VF	22	Group 1	7	6 (86%)	
		Group 2	15	7 (47%)	0.14
Syncope alone	35	Group 1	12	6 (50%)	
		Group 2	23	3 (13%)	0.016
Asymptomatic	36	Group 1	15	3 (20%)	
		Group 2	21	0 (0%)	0.039

The p value was calculated according to the log-rank test.
VF = ventricular fibrillation.

patients who had ST-segment augmentation at early recovery had a higher incidence of cardiac events than patients who did not. These data suggested the potential utility of exercise testing to predict cardiac events for patients with BrS who have had previous episodes of only syncope but not VF or who have had no symptoms.

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Key Words: Brugada syndrome ▪ exercise testing ▪ ST-segment elevation.

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Risk for Life-Threatening Cardiac Events in Patients With Genotype-Confirmed Long-QT Syndrome and Normal-Range Corrected QT Intervals

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Objectives This study was designed to assess the clinical course and to identify risk factors for life-threatening events in patients with long-QT syndrome (LQTS) with normal corrected QT (QTc) intervals.

Background Current data regarding the outcome of patients with concealed LQTS are limited.

Methods Clinical and genetic risk factors for aborted cardiac arrest (ACA) or sudden cardiac death (SCD) from birth through age 40 years were examined in 3,386 genotyped subjects from 7 multinational LQTS registries, categorized as LQTS with normal-range QTc (≤440 ms [n = 469]), LQTS with prolonged QTc interval (>440 ms [n = 1,392]), and unaffected family members (genotyped negative with ≤440 ms [n = 1,525]).

Results The cumulative probability of ACA or SCD in patients with LQTS with normal-range QTc intervals (4%) was significantly lower than in those with prolonged QTc intervals (15%) ($p < 0.001$) but higher than in unaffected family members (0.4%) ($p < 0.001$). Risk factors ACA or SCD in patients with normal-range QTc intervals included mutation characteristics (transmembrane-missense vs. nontransmembrane or nonmissense mutations: hazard ratio: 6.32; $p = 0.006$) and the LQTS genotypes (LQTS type 1:LQTS type 2, hazard ratio: 9.88; $p = 0.03$; LQTS type 3:LQTS type 2, hazard ratio: 8.04; $p = 0.07$), whereas clinical factors, including sex and QTc duration, were associated with a significant increase in the risk for ACA or SCD only in patients with prolonged QTc intervals (female age >13 years, hazard ratio: 1.90; $p = 0.002$; QTc duration, 8% risk increase per 10-ms increment; $p = 0.002$).

Conclusions Genotype-confirmed patients with concealed LQTS make up about 25% of the at-risk LQTS population. Genetic data, including information regarding mutation characteristics and the LQTS genotype, identify increased risk for ACA or SCD in this overall lower risk LQTS subgroup. (J Am Coll Cardiol 2011;57:51–9) © 2011 by the American College of Cardiology Foundation

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Abbreviations and Acronyms

ACA = aborted cardiac arrest
ECG = electrocardiographic
LQTS = long-QT syndrome
LQT1 = long-QT syndrome type 1
LQT2 = long-QT syndrome type 2
LQT3 = long-QT syndrome type 3
QTc = corrected QT interval
SCD = sudden cardiac death

Congenital long-QT syndrome (LQTS) is an inherited channelopathy characterized by a prolonged corrected QT interval (QTc) at rest that is associated with an increased predisposition for polymorphic ventricular arrhythmias and sudden cardiac death (SCD) in young subjects without structural heart disease (1). To date, more than 500 mutations have been identified in 12 LQTS-susceptibility genes, with the long-QT syndrome type 1 (LQT1), long-QT syndrome type 2 (LQT2), and long-QT syndrome type 3 (LQT3) genotypes constituting more than 95% of genotype-positive LQTS and approximately 75% of all LQTS (2). Risk assessment in affected patients with LQTS relies primarily on a constellation of electrocardiographic (ECG) and clinical factors, including QTc interval and age-sex interactions (3–6). In addition, there is increasing evidence that genetic information and the molecular and cellular properties of the LQTS-causative mutation may identify subjects with increased risk for cardiac events (7–10). Despite these recent advances, however, currently there are limited data regarding the clinical course and risk factors for life-threatening events in patients with LQTS with normal resting QTc values, so-called silent mutation carriers, concealed LQTS, or normal–QT interval LQTS.

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In the present study we used combined data from 7 national LQTS registries to: 1) compare the clinical courses of patients with LQTS and normal-range QTc intervals to those of patients with prolonged QTc intervals and of genotype-negative unaffected family members; and 2) identify specific clinical and genetic risk factors for life-threatening cardiac events in patients with LQTS with normal-range QTc intervals.

Methods

Study population. The study population comprised 3,386 genotyped subjects drawn from the Rochester, New York, enrolling center (center 1) of the International LQTS Registry (n = 2,630), the Netherlands LQTS Registry (n = 391), and the Japanese LQTS Registry (n = 205), as well as from data submitted by other investigators specifically for this collaborative mutation analysis project from Denmark (n = 90), Italy (n = 28), Israel (n = 25), and Sweden (n = 17). Patients were derived from 552 proband-identified *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3) families. The proband in each family had otherwise unexplained, diagnostic QTc prolongation or experienced LQTS-related symptoms. Patients were excluded from the study if they had: 1) >1 LQTS identified mutation (n = 70); 2) Jervell and Lange-Nielsen syndrome with deafness and 2 *KCNQ1* mutations or 1 known *KCNQ1* mutation and congenital deafness (n = 2); and 3) no identified mutation on genetic testing with prolonged QTc interval (>440 ms [n = 428]).

Data collection and end point. Routine clinical and rest ECG parameters were acquired at the time of enrollment in each of the registries. Measured parameters on the first recorded electrocardiogram included QT and R-R intervals in milliseconds, with QT interval corrected for heart rate using Bazett's (11) formula. Clinical data were collected on prospectively designed forms with information on demographic characteristics, personal and family medical histories, ECG findings, therapies, and events during long-term follow-up. Data common to all LQTS registries involving genetically tested subjects were electronically merged into a common database for the present study. In addition, information regarding QT interval–prolonging medications and triggers for cardiac events was collected through a specific questionnaire for patients enrolled the U.S. portion of the registry.

The primary end point of the study was the occurrence of a first life-threatening cardiac event, comprising aborted cardiac arrest (ACA; requiring external defibrillation as part of the resuscitation or internal defibrillation in patients with implantable cardioverter-defibrillators) or LQTS-related SCD (abrupt in onset without evident cause, if witnessed, or death that was not explained by any other cause if it occurred in a nonwitnessed setting such as sleep). In the multivariate models, follow-up was censored at age 41 years to avoid the influence of coronary disease on the occurrence of cardiac events. We also evaluated a secondary end point that included the occurrence of a first cardiac event of any type during follow-up (comprising syncope [defined as transient loss of consciousness that was abrupt in onset and offset], ACA, or SCD).

Phenotype characterization. For the purpose of this study, the QTc interval was categorized as normal range (≤440 ms) or prolonged (>440 ms) according to accepted criteria for the phenotypic definition of LQTS (12). Using this definition, the study population were categorized into 3 genotype and QTc subgroups: 1) LQTS with normal-range QTc interval (n = 469), comprising patients identified to have LQT1 to LQT3 mutations with QTc intervals ≤440 ms; 2) LQTS with prolonged QTc interval (n = 1,392), comprising patients with LQT1 to LQT3 mutations with QTc intervals >440 ms; and 3) unaffected family members (n = 1,525), comprising registry subjects from genotype-positive proband-identified families who were genetically tested and found to be negative for the LQTS-associated mutation, with QTc intervals ≤440 ms (i.e., genetically and phenotypically unaffected family members).

Genotype characterization. The *KCNQ1*, *KCNH2*, and *SCN5A* mutations were identified with the use of standard genetic tests performed in academic molecular genetics laboratories, including the Functional Genomics Center, University of Rochester Medical Center, Rochester, New York; Baylor College of Medicine, Houston, Texas; Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, Minnesota; Boston Children's Hospital, Boston, Massachusetts; the Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; the Department of Clinical Genetics, Academic Medical Center, Amsterdam, the Netherlands; and the Molecular Cardiology Laboratory, Policlinico S. Matteo and University of Pavia, Pavia, Italy.

Genetic alterations of the amino acid sequence were characterized by location and by the type of the specific mutation. The transmembrane region of each of the 3 LQTS channels was defined as: 1) amino acid residues from 120 through 355 in the *KCNQ1*-encoded Kv7.1 channel (S1 to S6 region); 2) amino acid residues from 398 through 657 (S1 to S6 region) in the *KCNH2*-encoded Kv11.1 channel; and 3) amino acid residues 129 through 417, 713 through 940, 1201 through 1470, and 1523 through 1740 in the *SCN5A*-encoded Nav1.5 channel (13). On the basis of prior studies that demonstrated the functional and clinical importance of missense mutations that are located in the transmembrane region of these LQTS-associated channels (9,10), mutation categories were pre-specified in the primary analysis as transmembrane-missense (mutations of the missense type in any of the 3 transmembrane regions described previously) versus nontransmembrane or nonmissense (i.e., any other identified LQT1 to LQT3 mutation that was not transmembrane-missense).

Statistical analysis. The clinical characteristics of study patients were compared by genotype and QTc categories using chi-square tests for categorical variables and *t* tests and Mann-Whitney-Wilcoxon tests for continuous variables. The Kaplan-Meier estimator was used to assess the time to a first life-threatening event and the cumulative event rates by risk groups and risk factors, and groups were compared using the log-rank test.

Cox proportional hazards regression analysis was carried out in the total study population and separately in the subset of patients with genotype-positive LQTS. Pre-specified covariates in the total population model included the 3 genotype and QTc categories, sex, and time-dependent beta-blocker therapy. The models comprising genotype-positive patients included the following pre-specified covariates: QTc category (normal range [≤440 ms] vs. prolonged [>440 ms]), the LQT1 to LQT3 genotypes, mutation location and type, sex, QTc duration (assessed both as a continuous measure [per 10-ms increase] and as a categorical covariate [dichotomized at the median value of each QTc category and assessed in separate models]), time-dependent beta-blocker therapy, and a family history of SCD in a first-degree relative. The effect of each covariate on outcome in each QTc category (i.e., in patients with LQTS with normal-range and prolonged QTc intervals) was assessed using interaction-term analysis, with interactions tested 1 at a time. Estimates of predictor hazard ratios in the separate normal and prolonged QTc categories were obtained using these interactions. To avoid violation of the proportional hazards assumption due to sex-risk crossover during adolescence, we used an age-sex interaction term in the multivariate models.

Because almost all the subjects were first-degree and second-degree relatives of probands, the effect of lack of independence between subjects was evaluated in the Cox model with grouped jackknife estimates for family membership (14). All grouped jackknife standard errors for the covariate risk factors fell within 3% of those obtained from the unadjusted Cox model, and therefore only the Cox model findings are reported. The statistical software used for the analyses was SAS version 9.20 (SAS Institute Inc., Cary, North Carolina). A 2-sided significance level of 0.05 was used for hypothesis testing.

Results

The spectrum and number of LQT1-associated, LQT2-associated, and LQT3-associated mutations by the pre-specified location and type categories are presented in Online Table 1. Totals of 100, 177, and 41 different mutations were identified in the *KCNQ1*-encoded Kv7.1, *KCNH2*-encoded Kv11.1, and *SCN5A*-encoded Nav1.5 ion channels, respectively. Study patients with identified LQTS mutations exhibited a very wide QTc interval distribution (Fig. 1), ranging from a minimum of 350 ms to a maximum of 800 ms (mean 450 ± 56 ms; median 440 ms; interquartile range: 410 to 480 ms). QTc distribution was similar among the 3 LQTS genotypes. Four hundred sixty-nine LQTS mutation–positive patients exhibited normal-range QTc intervals, constituting 25% of identified cases.

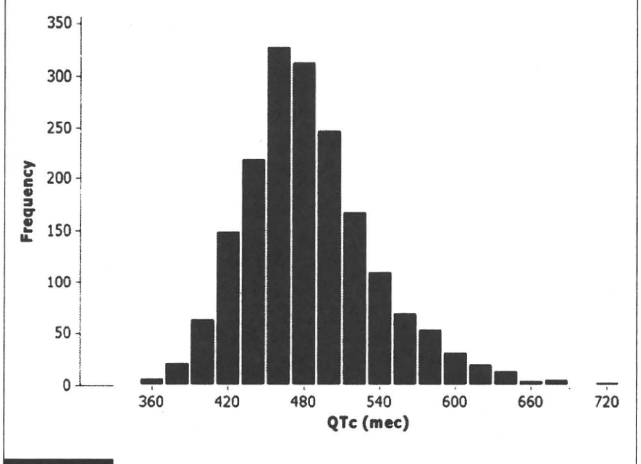

Figure 1 **Distribution of QTc Interval Duration in Genotype-Positive Patients With LQTS**

Distribution of corrected QT (QTc) interval durations in genotype-positive study patients. LQTS = long-QT syndrome.

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Table 1 Baseline and Follow-Up Characteristics of the Study Population by Genotype-Phenotype

Characteristic	Unaffected Family Members (n = 1,525)	Patients With LQTS With Normal-Range QTc Intervals (n = 469)	Patients With LQTS With Prolonged QTc Intervals (n = 1,392)
Female	52%	48%	61%*†
Family history of SCD	8%	12%	19%*†
QTc interval (ms)			
Mean ± SD	412 ± 22	419 ± 20	501 ± 48
Median (IQR)	420 (400–430)	420 (410–440)	490 (470–520)
Proband	8%	8%	29%*†
RR interval (ms)			
Mean ±SD	793 ± 221	888 ± 236	848 ± 214*†
Median (IQR)	800 (640–930)	900 (740–1,040)	840 (700–1,000)*†
Genotype			
LQT1	NA	40%	39%
LQT2	NA	45%	47%
LQT3	NA	16%	14%
Mutation: TM-MS			
Overall	NA	35%	43%
LQT1	NA	45%	61%
LQT2	NA	16%	29%†
LQT3	NA	64%	31%†
Therapies			
Beta-blockers	6.2%	38%	54%*†
Pacemaker	0.3%	0.6%	5%*†
LCSD	0.1%	0.2%	1.4%*†
ICD	0.6%	6%	14%*†
Events			
Syncope	10%	21%	40%*†
ACA	0.2%	1.3%	8.4%*†
SCD	0.1%	1.5%	4.4%*†
ACA/SCD‡§	0.3%	2.8%	11.3%*

*$p < 0.05$ for the comparison among the 3 genotyped categories. †$p < 0.05$ for the comparison between genotype-positive patients with QTc intervals ≤440 ms and genotype-positive patients with QTc intervals >440 ms. ‡Appropriate ICD shocks constituted 0.04% of ACAs in genotype-positive patients with QTc intervals ≤440 ms and 1.4% of ACAs in genotype-positive patients with QTc intervals >440 ms. §Only the first event for each patient was considered.

ACA = aborted cardiac arrest; ICD = implantable cardioverter-defibrillator; IQR = interquartile range; LCSD = left cardiac sympathetic denervation; LQT1 = long-QT syndrome type 1; LQT1 = long-QT syndrome type 2; LQT3 = long-QT syndrome type 3; LQTS = long-QT syndrome; MS = missense; NA = not applicable; QTc = corrected QT; SCD = sudden cardiac death; TM = transmembrane.

The clinical characteristics of the total study population by genotype and QTc subgroup are shown in Table 1. The frequency of probands (defined in the registry as the first person in a family, living or deceased, identified to have LQTS by the enrollment center) was highest in patients with prolonged QTc intervals, whereas most patients with normal-range QTc intervals (92%) were asymptomatic at the time of genetic testing. The frequency of female subjects was similar between the unaffected subjects and patients with LQTS with normal-range QTc intervals and higher in patients with prolonged QTc intervals. In mutation carriers, the frequency of the 3 main LQTS genotypes was similar between patients with and without prolonged QTc intervals. However, patients with LQT1 and LQT2 with prolonged QTc intervals had a higher frequency of transmembrane-missense mutations compared with the corresponding genotype carriers who had normal-range QTc intervals. LQTS-related therapies were administered to a significantly higher frequency of patients with prolonged QTc intervals than to subjects in the other 2 subgroups (Table 1).

Clinical course by genotype and QTc subgroup. Kaplan-Meier survival analysis (Fig. 2) demonstrated a relatively low rate of ACA or SCD in patients with LQTS with normal-range QTc intervals (4% at age 40 years and 10% at age 70 years). Event rates were significantly higher in patients with prolonged QTc intervals (15% and 24% at age 70 years; log-rank $p < 0.001$ for the comparison with the normal-range QTc subgroup) and significantly lower in unaffected family members (0.4% and 1% at age 70 years; log-rank $p < 0.001$ for the comparison with the normal-range QTc subgroup and for the overall difference among the 3 subgroups). Notably, life-threatening events in patients with normal-range QTc intervals occurred mostly after age 10 years, whereas patients with prolonged QTc intervals exhibited an earlier onset of life-threatening events (Fig. 2).

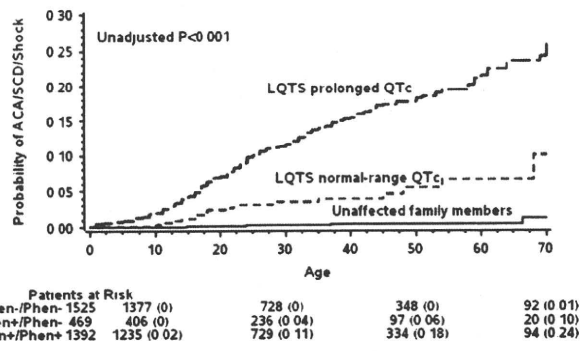

Figure 2 **Rate of ACA or SCD by Genotype and QTc Category**

Kaplan-Meier cumulative probabilities of aborted cardiac arrest (ACA) and sudden cardiac death (SCD) by genotype and corrected QT (QTc) subgroup.
LQTS = long-QT syndrome.

After multivariate adjustment for sex, time-dependent beta-blocker therapy, and a family history of SCD in a first-degree relative, patients with LQTS with normal-range QTc intervals were shown to have a significant 72% ($p < 0.001$) lower risk for ACA or SCD compared with patients with prolonged QTc intervals but also exhibited a >10-fold increase in the risk for life-threatening events compared with unaffected family members (Table 2). Histories of syncope were present in 62% of patients with LQTS with normal-range QTc intervals who had life-threatening events during follow-up. Accordingly, when the composite secondary end point of a first cardiac event of any type was assessed (comprising mainly non-life-threatening syncopal episodes), patients with normal-range QTc intervals were consistently shown to be at a lower risk compared with those with prolonged QTc intervals (hazard ratio [HR]: 0.47; 95% confidence interval [CI]: 0.33 to 0.59; $p < 0.001$) and at a higher risk compared with unaffected family members (HR: 5.20; 95% CI: 4.19 to 6.44; $p < 0.001$).

Risk factors for ACA or SCD in patients with LQTS with and without prolonged QTc intervals. Interaction-term analysis demonstrated significant differences in risk factors for life-threatening events between the 2 LQTS subgroups (Table 3). In patients with normal-range QTc intervals, the LQT1 and LQT3 genotypes were associated with respective 10- and 8-fold increases in the risk for life-threatening events compared with the LQT2 genotype. In contrast, in patients with prolonged QTc intervals, the LQT1 genotype was associated with one-half the risk of the LQT2 genotype ($p = 0.002$), with a statistically significant genotype–by–QTc subgroup interaction ($p = 0.006$) (Table 3, first row), and the LQT3 genotype showed a similar risk to the LQT2 genotype, without a statistically significant genotype–by–QTc subgroup interaction (Table 3, second row).

The location and type of the LQTS mutation were shown to be significant risk factors for ACA or SCD in patients with normal-range QTc intervals. In this LQTS subset, transmembrane-missense mutations were associated with a pronounced >6-fold ($p = 0.006$) increase in the risk for ACA or SCD compared with nontransmembrane or nonmissense mutations. In contrast, in patients with prolonged QTc intervals, transmembrane-missense mutations were not independently associated with outcomes (Table 3, third row). Notably, when the secondary end point of cardiac events of any type was assessed, transmembrane-missense mutations were shown to be an independent risk factor in both LQTS subgroups (normal-range QTc interval, HR: 1.71; 95% CI: 1.16 to 2.34; prolonged QTc interval, HR: 1.39; 95% CI: 1.17 to 1.65).

Consistent results demonstrating an association between transmembrane-missense mutations and the risk for ACA or SCD in patients with normal-range QTc intervals were shown when the reference group (comprising nontransmembrane or nonmissense mutations) was further divided into 3 subcategories, including nonmissense mutations in the transmembrane region, missense mutations in the nontransmembrane region, and nonmissense mutations in the nontransmembrane region (HR >4.0 for all 3 comparisons). Accordingly, patients with normal-range QTc intervals with transmembrane-missense mutations experienced a relatively high rate of ACA or SCD during follow-up (9% at age 40 years and 21% at age 70 years), whereas patients with normal-range QTc intervals with other mutations had a very low event rate (1% at age 40 years and 5% at age 70 years; log-rank p for overall difference = 0.005) (Fig. 3A). In contrast, in patients with prolonged QTc intervals, there was no statistically significant difference in the rate of ACA or SCD between the 2 mutation categories (16% and 14% at 40 years, respectively, $p = 0.18$) (Fig. 3B).

Clinical and ECG factors, including sex and QTc duration, were shown to be associated with a significant increase in the risk for ACA or SCD only in patients with prolonged QTc intervals (Table 3, rows 4 to 6). In contrast, in patients

Table 2 **Multivariate Analysis: Risk for ACA or SCD Among the 3 Genotype and QTc Categories***

Genotype and QTc Subgroup	HR	95% CI	p Value
LQTS with prolonged QTc interval vs. unaffected family members	36.53	13.35–99.95	<0.001
LQTS with normal-range QTc interval vs. unaffected family members	10.25	3.34–31.46	<0.001
LQTS with normal-range QTc interval vs. LQTS with prolonged QTc interval	0.28	0.16–0.49	<0.001

*Model also adjusted for sex (female age ≥13 years) and time-dependent beta-blocker therapy.
CI = confidence interval; HR = hazard ratio; other abbreviations as in Table 1.

Table 3 Risk Factors for ACA or SCD in Patients With LQTS by QTc Interval Category*

	LQTS and Normal-Range QTc Interval		LQTS and Prolonged QTc Interval		
Variable	HR (95% CI)	p Value	HR (95% CI)	p Value	p Value for Interaction
Genotype					
LQT1 vs. LQT2	9.88 (1.26–37.63)	0.03	0.53 (0.35–0.79)	0.002	0.006
LQT3 vs. LQT2	8.04 (0.85–36.03)	0.07	1.07 (0.70–1.63)	0.77	0.08
Mutation location and type					
TM-MS vs. non-TM-MS	6.32 (1.71–23.33)	0.006	1.24 (0.88–1.76)	0.22	0.02
Sex					
Female age >13 yrs vs. male age >13 yrs	1.32 (0.42–4.17)	0.64	1.90 (1.26–2.86)	0.002	0.53
QTc interval (ms)					
Per 10-ms increase	1.20 (0.81–1.78)	0.35	1.08 (1.05–1.10)	0.001	0.58
≥Median vs. <median†	1.03 (0.36–2.98)	0.95	2.96 (2.06–4.26)	0.001	NA

*Cox proportional hazards regression modeling was carried out in models that included all patients with genotype-positive LQTS (n = 1,861). Covariates in the models included QTc category (≤ 440 ms vs. > 440 ms), genotype, mutation location and type, sex, QTc interval (assessed as a continuous measure [per 10-ms increase]), time-dependent beta-blocker therapy, and a family history of SCD; the effect of each covariate in patients with normal-range (≤ 440 ms) and those with prolonged (>440 ms) QTc intervals was assessed by interaction-term analysis, with interactions tested 1 at a time. Estimates of predictor hazard ratios in the separate normal-range and prolonged QTc interval groups were obtained using these interactions. Virtually identical results for all pre-specified risk factors were also obtained from the models that did not include appropriate ICD shocks as part of the composite end point. †Results were obtained from separate models that assessed the risk associated with QTc values greater than or equal to the median in patients with LQTS with normal-range QTc intervals (median 420 ms) and prolonged QTc intervals (median 500 ms).

Abbreviations as in Tables 1 and 2.

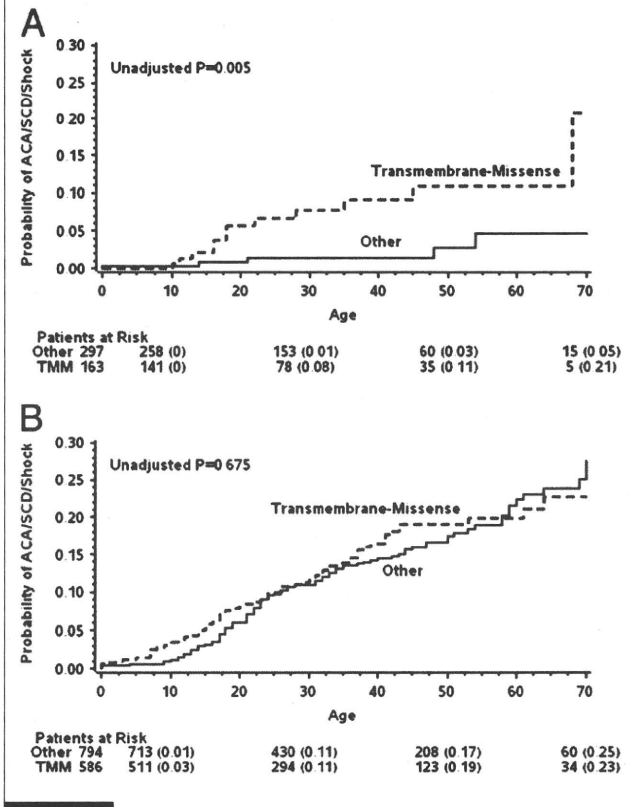

Figure 3 Rate of ACA or SCD in Patients With Normal-Range and Prolonged QTc Intervals by Mutation Location and Type

Kaplan-Meier cumulative probabilities of aborted cardiac arrest (ACA) and sudden cardiac death (SCD) by mutation location and type in patients with long-QT syndrome (LQTS) with **(A)** corrected QT (QTc) intervals ≤440 ms and **(B)** QTc intervals >440 ms.

with normal-range QTc intervals, sex was not a significant risk factor, and QTc duration was not independently associated with a significant increase in the risk for ACA or SCD when assessed as a continuous measure or when dichotomized at the median value (≥420 ms).

As suggested previously (15), the presence of a family history of SCD in any first-degree relative was not shown to be an independent predictor of ACA or SCD in patients with either normal-range QTc intervals (HR: 0.89; 95% CI: 0.63 to 1.25; p = 0.50) or prolonged QTc intervals (HR: 1.40; 95% CI: 0.32 to 6.17; p = 0.65) after adjustment for genetic and clinical factors.

Beta-blocker therapy was administered to 38% of patients who had normal-range QTc intervals compared with 54% of the patients who had prolonged QTc intervals (p < 0.001) (Table 1). Treatment with beta-blockers was associated with an overall significant 25% reduction in the risk for ACA or SCD in the total study population (95% CI: 0.70 to 0.80; p < 0.001), with similar effects in patients with normal-range QTc intervals and those with prolonged QTc intervals (p for beta-blocker–by–LQTS subset interaction = 0.45).

Characteristics of fatal or near-fatal cases with a normal-range QTc intervals. The characteristics of patients with normal-range QTc intervals who experienced ACA or SCD during follow-up are shown in Table 4. The mean age at occurrence of the lethal or near-lethal event in this population was 25.9 ± 4.5 years. Nine of the patients (53%) who experienced events were women, and 4 (24%) were treated with beta-blockers are the time of the events. In patients with normal-range QTc intervals with available data regarding therapies and triggers at the time of the events, none were reported as being treated with a QT interval–prolonging drugs at the time of ACA or SCD, and the majority of the lethal or near-lethal events were not associated with exercise or arousal triggers (Table 4).

Table 4 Characteristics of ACA and SCD Cases With Normal-Range QTc Intervals

Case	Event	Event Age (yrs)	Female	QTc Interval (ms)	BB†	LCSD‡	PM‡	ICD‡	QT PD	Trigger*	Genotype	Mutation Location and Type
1	SCD	0.5	–	390	–	–	–	–	–	NA	LQT3	Non-TM-MS
2	ACA	10		430	–	–	–	–	–	Exercise	LQT1	TM-MS
3	ACA/shock	11	+	400	–	–	–	+	–	Non-E/A	LQT1	TM-MS
4	SCD	13	–	440	+	–	–	–	NA	NA	LQT1	TM-MS
5	ACA	14	–	410	–	–	–	–	–	Exercise	LQT1	Non-TM-MS
6	SCD	16	+	420	–	–	–	–	–	Non-E/A	LQT3	TM-MS
7	ACA	16	+	440	–	–	–	–	–	Arousal	LQT1	TM-MS
8	SCD	18	–	430	+	–	–	–	–	Non-E/A	LQT1	TM-MS
9	ACA	18	+	410	–	–	–	–	–	Exercise	LQT1	TM-MS
10	SCD	21	+	380	–	–	–	–	–	Arousal	LQT2	Non-TM-MS
11	SCD	22	–	440	–	–	–	–	NA	NA	LQT1	TM-MS
12	SCD	28	–	410	–	–	–	–	–	Exercise	LQT1	TM-MS
13	ACA	35	+	420	–	–	–	–	–	Non-E/A	LQT3	TM-MS
14	ACA	46		440		–	–	–	NA	NA	LQT2	TM-MS
15	SCD	48	–	430	+	–	–	–	–	Non-E/A	LQT2	Non-TM-MS
16	ACA	54	+	420	–	–	–	–	–	Non-E/A	LQT3	Non-TM-MS
17	SCD	69	–	380	–	–	–	–	NA	NA	LQT1	TM-MS

*Data regarding triggers for cardiac events and treatment with QT interval–prolonging medications were available for study patients who were enrolled in the U.S. portion of the International LQTS Registry. †At time of event. ‡Implanted or performed before event.

BB = beta-blocker therapy; E/A = exercise/arousal trigger for event; NA = not available; PM = pacemaker; QT PD = QT interval–prolonging drug; other abbreviations as in Tables 1 and 2.

Discussion

In this study, we assessed the clinical courses and risk factors for life-threatening events in LQTS patients with genetically-confirmed LQTS who do not exhibit the disease's phenotypic hallmark of QT interval prolongation, otherwise referred to as concealed LQTS, normal–QT interval LQTS, or genotype-positive/ECG phenotype–negative LQTS. Similar to prior studies (16), we have shown that patients with LQT1 to LQT3 exhibit a wide QTc distribution, with approximately 25% having QTc intervals well within the normal range. The rate of ACA or SCD in patients with LQTS with normal-range QTc intervals was shown to be very low (4% from birth through age 40 years, corresponding to an approximate event rate of 0.13% per year). Comparatively, however, this very low risk subset of the LQTS population still exhibited a >10-fold increase in the risk for life-threatening events compared with genetically and phenotypically unaffected family members. Importantly, predictors of life-threatening events were shown to be significantly different between LQTS patients with and without prolonged QTc intervals. In the latter LQTS subgroup, genetic data, including knowledge of genotype and mutation characteristics, were shown to identify the risk for ACA or SCD, whereas in the former LQTS subgroup, female sex in the post-adolescence period and QTc duration were identified as the predominant risk factors for life-threatening events.

The clinical courses of patients with LQTS are variable because of incomplete penetrance (17). They are influenced by age, genotype, sex, environmental factors, therapy, and possibly other modifier genes (1–10). Recent studies from the International LQTS Registry that assessed the risk for life-threatening events in patients with LQTS have consistently demonstrated that ECG and clinical risk factors, including the QTc interval and age-sex interactions, identify increased risk in the LQTS population (3–5). These studies, however, included mainly phenotype-positive patients with LQTS with QTc intervals ≥ 450 ms. Thus, the effect of genetic data on outcomes in these studies was not statistically significant after adjustment for the ECG and clinical factors. The present study population, comprising 1,861 genetically confirmed patients with the LQT1 to LQT3 genotypes, extends the data derived from prior studies and demonstrates that risk factors for life-threatening events are significantly different between patients with LQTS with and without QTc prolongation. Consistent with prior studies, we have shown that in patients with LQTS who exhibit prolonged QTc durations, ECG information and clinical factors can be used to identify the risk for life-threatening events. In contrast, in mutation-positive subjects with normal-range QTc intervals, genetic factors, including knowledge of the LQTS genotypes and the mutation location and type, identified patients who were at an increased risk for ACA or SCD after adjustment for ECG and clinical data.

Sex was not a significant risk factor for cardiac events in patients with normal-range QTc intervals. Furthermore, patients with normal-range QTc intervals displayed a similar frequency of women as unaffected family members, whereas the frequency of women was significantly higher among patients with prolonged QTc intervals. These findings are in accordance with earlier evidence of longer QTc intervals in LQTS women than in men (18), resulting in a marked female predominance in phenotypically affected patients (3–5). The biologic basis for this sex difference might be the down-regulation of expression of cardiac potassium-channel genes by female

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sex hormones, which have been shown to prolong the QT interval in both congenital and drug-induced LQTS (19,20). These hormonal effects may explain the present findings of a lower frequency of LQTS women with normal-range QTc intervals.

Recent genotype-phenotype studies have shown that missense mutations located in the transmembrane region, which is responsible for forming the ion conduction pathway of the channel, are associated with a significantly higher risk for cardiac events compared with mutations that are located in other regions of the LQTS channel (9,10). The present study also shows that transmembrane-missense mutations are associated with a significantly higher risk for cardiac events of any type (predominated by syncopal episodes) in patients with LQTS with both normal-range and prolonged QTc intervals. However, our findings suggest that data regarding mutation characteristics are important for the assessment of life-threatening events (comprising ACA and SCD) mainly in patients with normal-range QTc intervals, in whom information derived from ECG and clinical data is more limited. In this LQTS subset, missense mutations located in the transmembrane region were shown to be associated with a >6-fold increase in the risk for life-threatening events and with a clinically meaningful rate of ACA or SCD (9%) from birth through age 40 years.

The mechanisms relating to the occurrence of life-threatening ventricular tachyarrhythmias in phenotype-negative patients with LQTS are not clear. In the present study, none of the patients with normal-range QTc intervals who experienced ACA or SCD took QT interval–prolonging medications at the time of the events. Furthermore, most events in patients with normal-range QTc intervals were not related to exercise or arousal triggers (Table 4). An ECG tracing from a patient with the LQT1 genotype who developed arrhythmic events despite a normal-range QTc interval showed spontaneous generation of polymorphic ventricular tachycardia without preceding extrasystolic pauses or sudden sinus rate acceleration (Fig. 4), possibly explaining the occurrence of ACA or SCD in study patients with normal-range QTc intervals who were treated with beta-blockers at the time of the events.

Study limitations. Most study patients did not undergo comprehensive genetic testing for all currently known mutations that may predispose to arrhythmic risk. Thus, it is possible that the coexistence of modifier genes affected the outcomes of patients with LQTS with normal-range QTc intervals who experienced life-threatening cardiac events. In addition, to provide an estimation of event rates among unaffected family members, we included in the control group subjects who were both genotype negative and also had normal-range QTc intervals (and excluded genotype-negative subjects with prolonged QTc intervals due to possible unidentified mutations in this subset). Therefore, the overall frequency of genotype-positive subjects in the total population may not represent the true penetrance of LQTS in affected families.

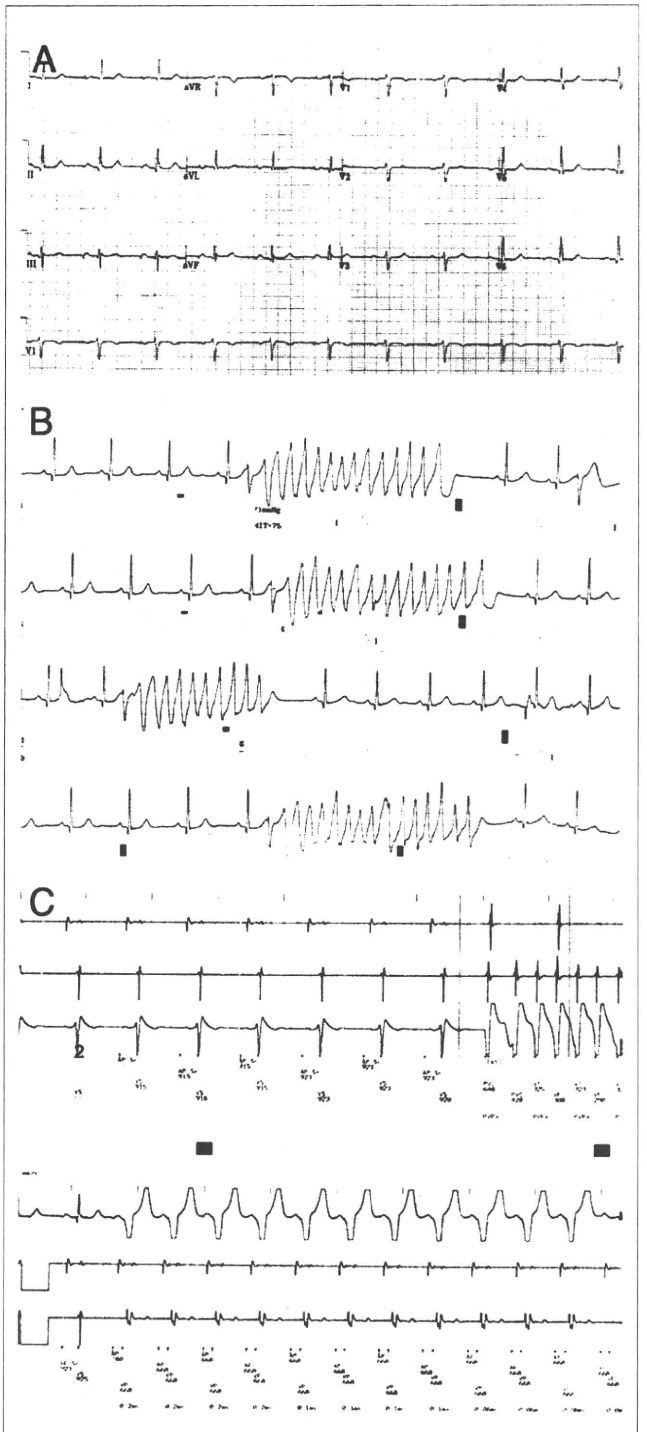

Figure 4 **Polymorphic Ventricular Tachycardia in a Patient With a Normal-Range QTc Interval**

Spontaneous generation of polymorphic ventricular tachycardia in a patient with long-QT syndrome type 1 with a normal-range corrected QT (QTc) interval. **(A)** The patient had a QTc duration of 410 ms on baseline electrocardiography. **(B)** Electrocardiographic tracing at the time of arrhythmic event demonstrates sinus rate with an RR interval of 1,000 ms without significant QT prolongation before the arrhythmia. **(C)** The patient was treated with nadolol and received an implantable cardioverter-defibrillator but continued to exhibit arrhythmic episodes that were recorded on implantable cardioverter-defibrillator interrogation.

The threshold value of 440 ms for the definition of a normal-range QTc in the present study was based on the diagnostic criteria for LQTS proposed by Schwartz et al. (12), which define a prolonged QTc interval as ≥450 ms in male patients and ≥460 ms in female patients. We chose to use a uniform approach by selecting 440 ms as the upper limit of normal rather than having separate phenotypic definitions for male and female patients. It should also be noted that 2.5% of infants and 10% to 20% of adults exceed this cutoff (21). Thus, the 440-ms value is not meant to suggest an LQTS diagnosis on its own.

Conclusions

The present study shows that patients with LQTS who exhibit normal-range QTc intervals constitute approximately 25% of the LQTS population and have a significantly lower risk for life-threatening events compared with phenotypically affected patients but also exhibit a significant increase in the risk of ACA or SCD compared with unaffected family members. Missense mutations in the transmembrane regions of the ion channels, mainly in patients with LQT1 and LQT3, were shown to identify patients with normal-range QTc intervals who have an increased risk for ACA or SCD. In contrast, increments in QTc duration were not shown to be significantly associated with increased risk for life-threatening events in this population. These findings suggest that: 1) risk assessment in phenotype-negative family members of LQTS probands should include genetic testing, because a positive genetic test result in a family member with a normal-range QTc interval implies an overall >10-fold increase in the risk for ACA or SCD compared with a negative test result in an unaffected family member; 2) genetic data may be used to identify phenotype-negative patients with LQTS who are at increased risk for fatal ventricular tachyarrhythmias independently of QTc duration; and 3) LQTS mutation–positive patients with normal-range QTc intervals who are identified as having increased risk for life-threatening events on the basis of genotype and mutation characteristics (i.e., LQT1 and LQT3 with transmembrane-missense mutations) should be carefully followed and receive a similar management strategy as phenotype-positive patients with LQTS, including avoidance of QT-prolonging medications (22), routine therapy with beta-blockers, and possibly implantable cardioverter-defibrillator therapy in those who remain symptomatic despite medical therapy. Conversely, patients with the lowest risk profile of already low risk, concealed LQTS (i.e., concealed LQT2 and non-transmembrane-missense LQT1 and LQT3) may represent the nominally near zero risk subpopulation(s) of LQTS in need of only preventative health recommendations such as QT drug avoidance.

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Key Words: corrected QT interval ▪ long-QT syndrome ▪ sudden cardiac death.

APPENDIX

For a table about *KCNQ1*, *KCNH2*, and *SCN5A* mutations by amino acid coding, frequency, location, and type, please see the online version of this article.

ANTICANCER RESEARCH *30*: 3833-3838 (2010)

Optimal Cutoff Points of CYFRA21-1 for Survival Prediction in Non-small Cell Lung Cancer Patients Based on Running Statistical Analysis

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Abstract. *Purpose: To determine pretreatment serum CYFRA21-1 levels as indicators of poor prognosis in patients with non-small cell lung cancer (NSCLC). Methods: 1,202 consecutive patients, diagnosed pathologically with NSCLC from January 1999 to December 2009, were entered in this study. To obtain optimal cutoff points of CYFRA21-1 for these endpoints, a running log-rank statistical method was applied. Results: The cutoff level for the maximum log-rank statistical value of one-year survival in patients with NSCLC was 18.0 ng/ml. These results could be applied to patients with squamous cell carcinoma. In multivariate analysis, elevated (>18.0 ng/ml) levels of CYFRA21-1 was confirmed as being an unfavourable prognostic factor. Conclusion: CYFRA21-1 assay has a clinical significance for identifying patients with poor prognosis among those with early and advanced NSCLC. Elevated serum CYFRA21-1 levels at the time of diagnosis may be a useful noninvasive marker for identifying the risk of early death from NSCLC.*

Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer cases, presenting as locally advanced in approximately 25-30% of cases and as metastatic disease in approximately 40-50% of cases. After radical treatment for seemingly localised disease, 20% of these patients develop an early distant relapse, probably due to systemic micrometastases that were present at the time of initial staging. One- and two-year survival rates are still used as the indices of survival for advanced NSCLC patients (1, 2).

Cytokeratin is a cytoskeletal structure expressed in epithelial cells, including bronchial epithelia (3). More than 20 subunits of cytokeratin are known and are expressed differently in several types of epithelia (4). Of these cytokeratins, cytokeratin 19 fragment (CYFRA21-1) levels in serum have already been evaluated as a useful tumour marker for non-small cell lung cancer (NSCLC) (5-18). Several studies have shown that elevated serum level of CYFRA21-1 is an independent prognostic factor of the risk of cancer death (8-18). The elevated serum levels of CYFRA21-1 could serve as a separate measure of biological aggressiveness and may have an important role in guiding treatment recommendations and in patient selection for clinical trials. However, regarding the use of CYFRA21-1 as a prognostic tool, neither the way of defining its cutoff points nor the use of multiple cutoff points for various survival conditions have been discussed in the published literature to date.

In evaluating the prognostic significance of elevated serum levels of CYFRA21-1, all the previous studies used manufacturer recommended cutoff levels of CYFRA21-1 for diagnosis of lung cancer (8-10, 12-18). Only one previous study analysed novel cutoff levels for poor prognosis (11). To calculate an optimal cutoff point, that study applied the receiver operation characteristic (ROC) method. This method, however, proved to be inapplicable because follow-up cases were not treated adequately in the survival analysis. In order to circumvent this weakness, a running log-rank statistic was calculated for each possible cutoff point on the basis of serum CYFRA21-1 level (19). The most optimal cutoff level was then determined as that corresponding to the maximum log-rank statistical value. As mentioned above, one- and two-year

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Key Words: Non-small cell lung cancer, cytokeratin, tumour marker, CYFRA21-1, prognosis, survival.

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survival rates are still used as the index of the survival for advanced NSCLC patients (1, 2). This study evaluated the CYFRA21-1 level which attained the maximum log-rank statistical value of one-, two-, and three-year survival time.

Patients and Methods

Study population. A retrospective study of serum CYFRA21-1 levels was conducted in patients with NSCLC. 1.202 consecutive patients, diagnosed pathologically with NSCLC at the Divisions of Respiratory Medicine and Thoracic surgery, Tsukuba University Hospital and Division of Respiratory Medicine, Tsukuba Medical Center Hospital from January 1999 to December 2009, were entered in this study. Staging procedure was performed for all patients according to TNM classification (20) using chest computed tomography (CT), brain magnetic resonance imaging (MRI), bone scan as well as ultrasonograpy and/or CT of the abdomen. Peripheral venous blood samples collected from patients with NSCLC were used for the CYFRA21-1 assay. The samples were stored at –30°C until use. This study was approved by the Institutional Review Boards of the participating hospitals.

Measurement of CYFRA21-1 levels. Blood sampling was performed within the 1-month period preceding therapy. The serum CYFRA21-1 level of all blood samples was determined using a chemiluminescent enzyme immunoassay method (Lumipulse I CYFRA, Fujirebio Inc, Tokyo, Japan). According to the manufacturer, the upper limit of the normal CYFRA21-1 level is 3.5 ng/ml. The assay was performed by technicians who had no clinical information regarding the samples.

Statistical methods. To obtain optimal cutoff levels of CYFRA21-1, running log-rank statistics was applied (19). Running log-rank statistics produced for each cutoff point of serum level of CYFRA21-1 were plotted against survival and tested for statistical significance *via* permutations of the data. In brief, the patients were divided into two groups: patients with serum CYFRA21-1 levels more than the cutoff point and those with serum CYFRA21-1 levels equal to or less than the cutoff point. Log-rank statistical values between the groups were calculated for each possible cutoff point on the basis of serum CYFRA21-1 up to level that covered 90% of the patients by 1.0 ng/ml increments. The CYFRA21-1 level which attained the maximum log-rank statistical value of one-, two- and three-year survival time between the two groups was evaluated as an optimal cutoff point. All statistical analyses were performed using the SAS 9.1.3 for Windows and R (R-2.7.0) computer packages (Cary, North Carolina, USA). *p*-values less than 0.05 were considered to be statistically significant.

Results

Table I shows the basic characteristics of NSCLC patients. Of the 1,202 NSCLC patients, 906 were men. Median age was 68 years (range: 21-94 years). Among them were 280 stage IA-IB and 728 stage IIIB and IV. Histological types were: 733 patients with adenocarcinoma (AD), 399 with squamous cell carcinoma (SQ), 60 with large cell carcinoma, and 10 with other types as defined by the WHO classification system.

Table I. *Characteristics of patients with non-small cell lung cancer.*

No. of patients	1.202
Age (year)	median: 68 range: 21-94
Gender	
Male	906 (75.4%)
Female	296 (24.6%)
Performance status	
0-1	963 (80.2%)
2-4	238 (19.8%)
Histology	
Adenocarcinoma	733 (61.0%)
Squamous cell carcinoma	399 (33.2%)
Large cell carcinoma	60 (5.0%)
Other	10 (0.8%)
Clinical stage	
IA-B	280 (23.3%)
IIA-B	58 (4.8%)
IIIA	136 (11.3%)
IIIB	285 (27.7%)
IV	443 (36.9%)

The serum CYFRA21-1 levels differed significantly according to clinical stage (Kruskal-Wallis test, p=0.0001). Maximum serum level of CYFRA21-1 was 562.1 ng/ml, and CYFRA21-1 level up to 20.0 ng/ml covered 94.2% of all 1.202 NSCLC patients.

As shown in Figure 1, the maximum log-rank statistical value of one-year survival in patients with NSCLC was 0.455, which gave an optimal cutoff point for CYFRA21-1 level of 18.0 ng/ml. The maximum log-rank statistical value of two- and three-year survival in patients with NSCLC was 0.485 and 0.489, which gave optimal cutoff points of 3.0 ng/ml and 1.0 ng/ml, respectively. In patients with AD, the maximum log-rank statistical value of one-year survival was 0.531 which gave an optimal cutoff point of 5.0 ng/ml. The maximum log-rank statistical value of two- and three-year survival in patients with AD was 0.524 and 0.504 which gave optimal cutoff points of 5.0 ng/ml and 5.0 ng/ml, respectively. In patients with SQ, the maximum log-rank statistical value of one-year survival was 0.476 which gave an optimal cutoff point of 18.0 ng/ml (Figure 2). The maximum log-rank statistical value of two- and three-year survival in patients with SQ was 0.665 and 0.723 which gave optimal cutoff points 1.0 ng/ml and 1.0 ng/ml, respectively.

Confirmation of the above results was performed using uni- and multivariate analysis. Elevated CYFRA21-1 levels (>18.0 ng/ml) in NSCLC patients showed statistical significance in survival (p=0.001, for uni- and multi-variate analysis). Elevated CYFRA21-1 levels (>18.0 ng/ml) in patients with SQ also conferred a poor prognosis (p=0.001 for uni- and multivariate analysis; data not shown).

Table II. *Uni- and multivariate analyses of prognostic factors in patients with non-small cell lung cancer.*

Factor	Uni-variate analysis (log-rank test) *p*-Value	Hazard ratio	Multivariate analysis (Cox's proportional hazard model) 95%CI	*p*-Value
Stage (IA-IIIA, IIIB-IV)	0.001	2.72	1.48-5.01	0.001
Smoking (smoker, non-smoker)	0.004	0.20	0.07-0.60	0.004
Performance status (0-1, 2-4)	0.001	2.43	1.43-4.14	0.001
CYFRA21-1 (≤18.0, >18.0 ng/ml)	0.001	2.02	1.14-3.58	0.001
Treatment (surgery, other)	0.001	1.59	0.81-3.12	0.175

95% CI: 95% Confidence interval.

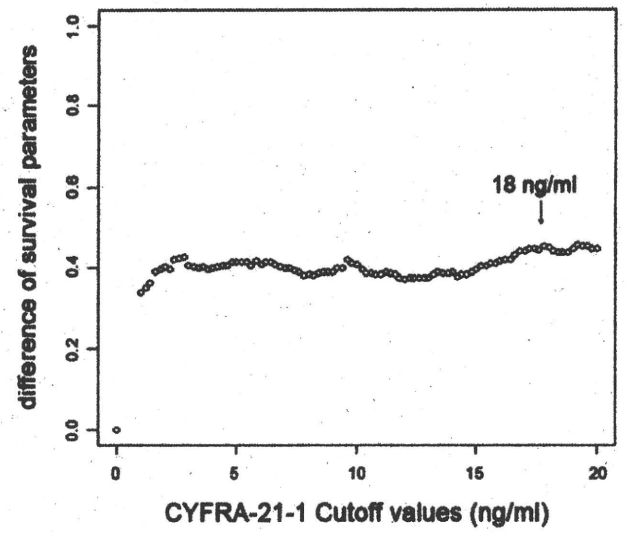

Figure 1. *Maximum log-rank statistical value of 1-year survival in patients with non-small cell lung cancer.*

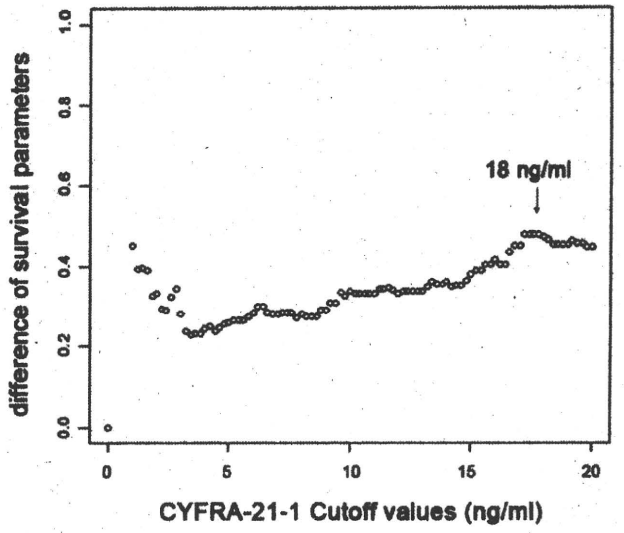

Figure 2. *Maximum log-rank statistical value of 1-year survival in patients with squamous cell carcinoma.*

Discussion

CYFRA21-1 is an immunometric assay measuring fragments of cytokeratin 19 and has been suggested to correlate with tumour burden and disease progression in NSCLC 12, 16). Prognostic significance of CYFRA21-1 has been discussed with conflicting results (5, 7, 11, 13, 14, 16-18). Most previous studies showed a prognostic significance for CYFRA21-1 (11, 13, 14, 16-18), while some studies did not (5, 7). In most of them, the prognostic significance of CYFRA21-1 was evaluated only for patients with resectable or resected NSCLC (9, 11, 18), or only for those with advanced disease (14, 17). In four large previous studies including more than one hundred NSCLC patients and which evaluated the prognostic significance of CYFRA21-1, the proportion of patients with early (stage IA-B) and advanced disease (stage IIIB-IV) was 8.7%-15.6%, and 62.7%-76.0%, respectively (6, 8, 10). However it would be preferable if the prognostic significance were evaluated in a large NSCLC patient population including both early and advanced clinical stages. Therefore, the present study evaluated serum CYFRA21-1 levels in NSCLC patients with operable stage as well as those with advanced or metastatic diseases. Above all, the major question dealt with in this study was whether patients who have elevated pretreatment serum CYFRA21-1 levels will have an unfavourable prognosis. If so, the next issue of this study was to determine pretreatment serum CYFRA21-1 levels that can be used as indicators of poor prognosis. Although currently the best predictor of outcome for patients with NSCLC is the TNM classification, the prognosis of patients within each stage of disease may vary considerably (1, 2). Early metastasis is a well-known feature of poor prognosis in potentially resectable NSCLC (21). The dissemination of malignant cells to distant organs *via* lymph nodes or blood vessels in NSCLCs can occur at an early stage of primary tumour growth and a significant number of early

staged NSCLC patients die of aggressive progression of the disease (21). In evaluating the prognostic significance of CYFRA21-1, most previous studies used the cutoff level that was recommended by the manufacturer of the assay kit, based on the specificity of the marker as the diagnostic tool to distinguish between lung cancer and normal individuals (8, 13-15). For example, Barlési *et al.* used a cutoff level of 3.5 ng/ml (14), and both Muley *et al.* and Merle *et al.* used a cutoff of 3.3 ng/ml (13, 15). In a large prospective study in 621 patients by Pujol *et al.*, the cutoff level was 3.6 ng/ml (10). Only Reinmuth *et al.* determined a cutoff CYFRA21-1 level using classification and regression tree survival analysis, resulting in a best predictive cutoff value of 3.57 ng/ml (11). The difference in the optimal CYFRA21-1 level for prognostic value between Reinmuth *et al.* (11) and the present study may be due to differences in the statistical analysis as well as the number of NSCLC patients and clinical stages of them evaluated. The analysis by Reinmuth *et al.* (11) included only 67 patients with completely resected NSCLC patients, and the proportion of stage IA-B and IIIB-IV cases were 85% and 0%, respectively. The present study included 1,202 patients, and the proportion of cases with early and advanced disease was 22.8% and 58.4%, respectively.

Recently, an optimal cutoff point for the diagnosis of lung cancer was determined using a ROC curve by choosing the value which attains the shortest length of (0, 1) point in the x-y plane, with the x axis representing the false positive proportion and the y axis representing the true positive proportion (22, 23). This method, however, does not cover the situation when censored data arise. To overcome this difficulty, running log-rank statistics (19) was applied in the present study. The value which attained the maximum statistic value between groups was naturally considered to be the best cutoff point for CYFRA2-1 levels. In this study, two notable results were found. Firstly, the maximum log-rank statistical value of one-year survival in NSCLC patients gave an optimal cutoff point of 18.0 ng/ml. The same cutoff level was also given by the maximum log-rank statistical value of one-year survival in SQ patients. Rather than the upper level for diagnosis, these findings could provide more important clinical information on identifying patients with poor prognosis. It seems reasonable that the optimal CYFRA21-1 point for poor prognosis, which was evaluated in this study, was higher than the manufacturer recommended cutoff value for diagnosis of NSCLC. Secondly, the cutoff levels for the maximum log-rank statistical value of two- and three-year survival in NSCLC, AD, and SQ patients were almost the same as the manufacturer recommended cutoff value for diagnosis. This result seemed to be somewhat unexpected; however, it may mean that if a patient had lung cancer with a higher CYFRA21-1 level than the manu-facturer recommended cutoff value, they would have a considerably unfavorable prognosis, irrespective of the histological type of NSCLC.

In spite of these significant findings, this study has limitations that need to be addressed before serum CYFRA21-1 can be used clinically at the time of diagnosis to predict subsequent mortality. Firstly, changes in serum CYFRA21-1 levels in serial measurement may be of great importance; however, this conclusion is only speculative, as there is no definite information on the serial measurements of CYFRA21-1 and changes in its level. Secondly, this study included NSCLC patients treated with various kinds of therapies. Thirdly, the development of these therapies may have influenced the prognosis of the patients. Therefore, these limitations may have affected the results of the present study.

In conclusion, elevated levels of CYFRA21-1 (>18.0 ng/ml) in NSCLC patients suggest a poor prognosis. In patients with SQ, CYFRA21-1 levels (>18.0 ng/ml) also predict poor prognosis. These findings suggest that CYFRA21-1 assay have a clinical significance for identifying patients with a poor prognosis among those with early and advanced NSCLC. In other words, elevated serum CYFRA21-1 levels at the time of diagnosis may be a useful noninvasive marker for identifying the risk of early death from NSCLC. A careful and large-sized clinical study may be necessary to corroborate these results.

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2．乳児突然死症候群における遺伝性不整脈の関与

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KEY WORDS
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遺伝性不整脈
先天性 QT 延長症候群
イオンチャネル

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はじめに

乳児突然死症候群（sudden infant death syndrome，SIDS）は，“それまでの健康状態および既往歴からその死亡を予測できず，死亡時の状況調査，死亡後の解剖検査によってもその原因を特定できない，原則として1歳未満の乳児の突然死”である（厚生労働省，乳幼児突然死症候群の診断の手引き）。その病名にあるようにSIDSは一種の症候群であり，複数の原因が含まれる可能性があるものの，生後2～4カ月に発生のピークがあり，睡眠時の死亡が多いこと，男児に多いこと，人種間で発生率に差があり，遺伝的素因の関与が示唆されることなどから，一つの疾患単位（entity）と考える研究者も多い。先進国の中で日本とオランダのSIDSの発生率はもっとも低く，出生1,000に対して約0.10～0.15である[1]（最多はニュージーランドで出生1,000に対して0.80）。しかし，日本でも平成年度半ばからSIDSが「不慮の事故」を抜いて乳児死因の第3位となり，平成19年の乳児死亡総数2,828のうち147（5.2%）がSIDSであった[2]。新生児死因順位ではSIDSは8位であり[2]，新生児期以降の乳児期前半に多いことを示している。うつぶせ寝や受動喫煙を避け，母乳を推奨するSIDS対策キャンペーンによりSIDSの数は世界的に著明に減少したものの，その詳細な原因はいまだに解明されず，完全に予防することは困難である。そのような背景のなか，分子生物学の進歩により，SIDS　の約10%において先天性

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QT 延長症候群（congenital long QT syndrome，LQTS）に代表される遺伝性不整脈（いわゆるイオンチャネル病：channelopathy）が関与していることを示唆する研究成果が相次いで発表された[3)~6)]。両者の関連の根拠は大きく分けて2つある。一つは救命された心停止（aborted sudden death）の症例で，蘇生後の心電図で QT 延長や torsade de pointes がみられ，遺伝子検査で LQTS が確定する症例が存在すること[7)]，もう一つは SIDS で死亡した乳児の死亡後の遺伝子検査で LQTS の遺伝子変異が検出される症例が存在することである[8)9)]。近年，特に後者の系統的な研究が行われるようになり[3)~6)]，一部の SIDS における遺伝性不整脈の関与の実態が明らかになりつつある。

Ⅰ．乳児突然死症候群における遺伝性不整脈（イオンチャネル病）の関与―その研究の歴史―

突然死が集積する家系の存在や，その家系の構成員が QT 延長やT波異常など共通の心電図所見を示すことは1960年代から知られ，これらの“遺伝する”不整脈が突然死の原因となっている可能性が指摘されていた。LQTS と SIDS との関連がはじめて報告されたのは1970年代のことである。Maron ら[10)]は SIDS 患児の親や蘇生された SIDS 児の心電図が QT 延長を示していることを初めて指摘した。Schwartz ら[11)]はこの関係を裏付ける前方視的研究として，1976～1994年に34,442人の新生児の心電図を生後3～4日に記録して1年間経過観察した。その結果，SIDS で死亡した24例の QT 時間はそれ以外の児よりも有意に長かった。QTc 値（$QT/\sqrt{RR}$）＞440msec を異常とすると，QTc が正常な児で SIDS が起こる率は0.037%，QTc が延長していた児で SIDS が起こる率は1.53%となり，Odds 比は41.3という高値を示した。この研究結果は新生児心電図スクリーニングの必要性や QTc＝440msec をカットオフ値とすることの是非を含め，多くの議論を巻き起こしたが，QT 延長（心筋再分極過程の延長）が SIDS と関連していることを初めて統計的に示した点で注目された。1990年代後半は LQTS が心筋イオンチャネルを構成するタンパクの異常であることが解明され，K^+ チャネル，Na^+ チャネル，Ca^{2+} チャネルなどをコードする遺伝子の変異が次々と発見された時代であり，Schwartz らの報告[11)]と相俟って突然死におけるイオンチャネルの関与の研究が発展する起点となった。

2000～2001年になると遺伝子検査によって LQTS と SIDS の関連を裏付ける報告が相次いだ。Schwartz ら[7)]は，ニアミス突然死を起こして救急搬送された乳児が，蘇生後の心電図で QT 延長と torsade de pointes を示し，さらに Na^+ チャネル遺伝子（*SCN5A*）に変異が認められたため，LQTS3型（LQT3）と診断された症例を報告した。また，死亡後の遺伝子診断で LQTS に特徴的な Na^+ チャネル（*SCN5A*）遺伝子や K^+ チャネル（*KCNQ1*）遺伝子の変異がみられた SIDS 症例が報告された[8)9)]。これらの症例は LQTS の遺伝子診断がなければ単に SIDS として登録されていた可能性が高い。

その後，SIDS の多数例（コホート）を対象として，死亡後に心筋などから抽出したゲノム DNA を用いて LQTS 関連遺伝子変異を検出する系統的研究が行われた。

Ackerman ら[3)]は SIDS 58例を対象として，*SCN5A* のミスセンス変異が2例に，K^+ チャネルの遺伝子変異が2例に認められたと報告した。Arnestad ら[4)]はノルウェーの SIDS 201例中，19例（9.5%）から LQTS 関連遺伝子の変異が検出されたと報告した。わが国でも Otagiri ら[6)]が42例の SIDS 症例