Clinical Aspects of Type-1 Long-QT Syndrome by Location, Coding Type, and Biophysical Function of Mutations Involving the KCNQ1 Gene

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Background—Type-1 long-QT syndrome (LQTS) is caused by loss-of-function mutations in the KCNQ1-encoded I_{Ks} cardiac potassium channel. We evaluated the effect of location, coding type, and biophysical function of KCNQ1 mutations on the clinical phenotype of this disorder.

Methods and Results—We investigated the clinical course in 600 patients with 77 different KCNQ1 mutations in 101 proband-identified families derived from the US portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82). The Cox proportional hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through age 40 years. The clinical characteristics, distribution of mutations, and overall outcome event rates were similar in patients enrolled from the 3 geographic regions. Biophysical function of the mutations was categorized according to dominant-negative (>50%) or haploinsufficiency (≤50%) reduction in cardiac repolarizing I_{Ks} potassium channel current. Patients with transmembrane versus C-terminus mutations (hazard ratio, 2.06; $P<0.001$) and those with mutations having dominant-negative versus haploinsufficiency ion channel effects (hazard ratio, 2.26; $P<0.001$) were at increased risk for cardiac events, and these genetic risks were independent of traditional clinical risk factors.

Conclusions—This genotype–phenotype study indicates that in type-1 LQTS, mutations located in the transmembrane portion of the ion channel protein and the degree of ion channel dysfunction caused by the mutations are important independent risk factors influencing the clinical course of this disorder. (***Circulation***. 2007;115:2481-2489.)

Key Words: electrocardiography ■ genetics ■ long-QT syndrome

The hereditary long-QT syndrome (LQTS) is characterized by prolonged ventricular repolarization on the ECG and arrhythmia-related syncope and sudden death.[1] Mutations in 1 or more of several ion channel genes are known to cause this disorder,[2] with mutations in the KCNQ1 gene causing the type-1 long-QT syndrome.[3,4] The KCNQ1 gene codes for the potassium channel protein responsible for the slow component of the delayed rectifier repolarizing current (I_{Ks}). Mutations involving this gene result in reduction of the repolarizing I_{Ks} current and lengthening of the QT interval.[3]

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Functional I_{Ks} channels result from the coassembly of 4 subunits into a tetrameric protein channel that is transported to the myocyte membrane. Each subunit contains 6 membrane-spanning domains (S1 to S6) flanked by amino (N)- and carboxyl (C)-terminus regions. Two distinct biophysical mechanisms mediate the reduced I_{Ks} current in patients with KCNQ1 mutations: (1) coassembly or trafficking defects in which mutant subunits are not transported

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properly to the cell membrane and fail to incorporate into the tetrameric channel, with the net effect being a ≤50% reduction in channel function (haploinsufficiency)[5]; and (2) formation of defective channels involving mutant subunits with the altered channel protein transported to the cell membrane, resulting in a dysfunctional channel having >50% reduction in channel current (dominant-negative effect).[6]

Limited prior studies involving relatively small numbers of patients with type-1 LQTS have been reported with conflicting results on the relationship between various KCNQ1 mutations and the clinical outcome.[7,8] We hypothesized that the location, coding type, and functional effect of the channel mutation would have important influence on the phenotypic manifestations and clinical course of patients with this disorder. To test this hypothesis, we investigated the clinical aspects of a large cohort of subjects having a spectrum of KCNQ1 mutations categorized by their location, coding type, and type of biophysical ion channel dysfunction.

Methods

Study Population

The study population of 600 subjects with genetically confirmed KCNQ1 mutations was derived from 101 proband-identified families with the type-1 LQTS disorder. The proband in each family had QTc prolongation not due to a known cause. The subjects were drawn from the US portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82). All subjects or their guardians provided informed consent for the genetic and clinical studies.

Phenotype Characterization

Routine clinical and ECG parameters were acquired at the time of enrollment in each of the registries. Follow-up was censored at age 41 years to avoid the influence of coronary disease on cardiac events. Measured parameters on the first recorded ECG included QT and R-R intervals in milliseconds, with QT corrected for heart rate by Bazett's formula. The QTc interval was expressed in its continuous form and categorized into 3 levels: <500, 500 to 530, and >530 ms. Clinical data were collected on prospectively designed forms with information on demographic characteristics, personal and family medical history, ECG findings, therapy, and end points during long-term follow-up. LQTS-related cardiac events included syncope, aborted cardiac arrest, or unexpected sudden death without a known cause. Data common to all 3 LQTS registries involving genetically identified patients with type-1 genotype were electronically merged into a common database for the present study.

Genotype Characterization

The KCNQ1 mutations were identified with the use of standard genetic tests performed in academic molecular-genetic laboratories including the Functional Genomics Center, University of Rochester Medical Center, Rochester, NY; Baylor College of Medicine, Houston, Tex; Mayo Clinic College of Medicine, Rochester, Minn; Boston Children's Hospital, Boston, Mass; Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; and Department of Clinical Genetics, Academic Medical Center, Amsterdam, Netherlands.

Genetic alterations of the amino acid sequence were characterized by location and by the specific mutation (missense, splice site, in-frame insertions/deletions, nonsense, stop codon, and frameshift). The transmembrane region of the KCNQ1-encoded channel was defined as the coding sequence involving amino acid residues from 120 through 355 (S5-pore-S6 region 285 to 355), with the N-terminus region defined before residue 120 and the C-terminus region after residue 355. Nineteen study patients had intron mutations predicted to disrupt the canonical splice-site domains. Fifty-one subjects died of sudden cardiac death at a young age but did not have genotype studies. These 51 subjects were assumed to have the same KCNQ1 mutation as other affected members of their respective family. Twelve subjects had 2 mutations, one in the KCNQ1 gene and a second mutation in another LQTS ion channel gene; these 12 subjects are described separately and are not included in any of the tables or outcome analyses. Subjects with Jervell and Lange-Nielsen syndrome with deafness and 2 KCNQ1 mutations as well as those with 1 known KCNQ1 mutation and congenital deafness are not included in the present study.

The biophysical function of the mutant channels was classified as having dominant-negative effect (>50% reduction in function) or haploinsufficiency (≤50% reduction in function) on the basis of the following: (1) cellular expression studies for those with missense (n=21) and nonsense (n=2) mutations reported in the literature, with the functional information derived exclusively from heterologous expression studies; and (2) assumed loss of function for identified nonsense, splice site, in-frame deletion, and frameshift mutations (n=10) that have not yet been functionally characterized. Forty-one missense mutations and the 3 intron mutations that have not been functionally reported in cellular expression studies were categorized as unknown in terms of type of functional perturbation.

Statistical Analysis

Differences in the univariate characteristics by specific groupings were evaluated by standard statistical methods. The primary end point was time to syncope, aborted cardiac arrest, or sudden death, whichever occurred first. The cumulative probability of a first cardiac event was assessed by the Kaplan-Meier method with significance testing by the log-rank statistic. The Cox proportional hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through age 40 years.[9] Stratified and unstratified Cox regression models, allowing for time-dependent covariates, were fit to estimate the adjusted hazard ratio of each factor as a predictor of first cardiac events. We observed that sex was not proportional as a function of age with crossover in risk at age 13 years on univariate Kaplan-Meier analysis. To relax the assumption of proportional hazards for sex over the entire age range, separate nonparametric baseline hazard functions were allowed for male and female subjects via the stratified Cox model; then, to summarize the sex effect, sex was modeled in an unstratified Cox model as a time-dependent covariate (via an interaction with time), allowing for different hazard ratios by sex before and after age 13 years.

Because almost all the subjects were first- 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.[10] 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.

Patients who died suddenly at a young age from suspected LQTS and who did not have an ECG for QTc measurement were identified in the Cox models as "QTc missing." Prespecified covariate interactions were evaluated. The influence of time-dependent β-blocker therapy (the age at which β-blocker therapy was initiated) on outcome was determined by adding this variable to the final Cox model containing the various covariates.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Total Study Population

The spectrum and number of KCNQ1 mutations by location, type of mutation, and functional effect are presented in Table 1, with the location frequency of the mutations presented diagrammatically in Figure 1. A total of 77 different KCNQ1

TABLE 1. KCNQ1 Mutations by Location and Coding, Type of Mutation, and Functional Effect

Location and Coding*	No. of Subjects†	Type of Mutation	Functional Effect‡
N-terminus			
M1V	1	Missense	Unknown
G57V	1	Missense	Unknown
Transmembrane			
W120C	2	Missense	Unknown
T144A	7	Missense	Unknown
A150fs/133 [del CT 451-452]	2	Frameshift	Haploinsufficiency
E160K	3	Missense	Unknown
G168R	44	Missense	Unknown
Y171X [513 C>G]	6	Nonsense	Haploinsufficiency
R174H	2	Missense	Unknown
A178P	5	Missense	Dominant-negative effect (a)
Y184S	18	Missense	Unknown
G185S	10	Missense	Unknown
G189E	2	Missense	Unknown
G189R	4	Missense	Dominant-negative effect (b)
R190Q	4	Missense	Haploinsufficiency (b, c)
L191fs/90 [del TGCGC 572-576]	8	Frameshift	Haploinsufficiency
R195fs/40 [del G 585]	2	Frameshift	Haploinsufficiency
S225L	13	Missense	Dominant-negative effect (d)
A226V	3	Missense	Unknown
R237P	1	Missense	Unknown
D242N	3	Missense	Unknown
R243C	13	Missense	Haploinsufficiency (e)
V254 mol/L	59	Missense	Dominant-negative effect (b, f)
R258C	1	Missense	Haploinsufficiency
R259C	1	Missense	Haploinsufficiency (g)
L266P	15	Missense	Unknown
G269D	35	Missense	Dominant-negative effect (h)
G269S	25	Missense	Haploinsufficiency (i)
L273F	6	Missense	Dominant-negative effect (a)
I274V	1	Missense	Unknown
S277L	3	Missense	Unknown
Y278H	2	Missense	Unknown
E284K	2	Missense	Unknown
G292D	3	Missense	Unknown
F296S	2	Missense	Unknown
G306R	2	Missense	Dominant-negative effect (b, j)
V310I	1	Missense	Unknown
T312I	14	Missense	Dominant-negative effect (a)
G314S	8	Missense	Dominant-negative effect (h, k, l, m)
Y315C	10	Missense	Dominant-negative effect (d, n)
Y315S	1	Missense	Dominant-negative effect (h, m)
D317G	3	Missense	Unknown
P320H	1	Missense	Unknown
T322 mol/L	2	Missense	Unknown
G325R	3	Missense	Unknown
delF340 [del CTT 1017-1019]	7	In-frame deletion	Haploinsufficiency
A341E	9	Missense	Dominant-negative effect (b)
A341V	20	Missense	Dominant-negative effect (o)

TABLE 1. Continued

Location and Coding*	No. of Subjects†	Type of Mutation	Functional Effect‡
P343S	1	Missense	Dominant-negative effect (p)
A344A/sp [1032 G>A]	27	Splice site	Haploinsufficiency
A344V	17	Missense	Unknown
S349W	15	Missense	Unknown
L353P	4	Missense	Unknown
C-terminus			
Q357H	3	Missense	Unknown
R360G	3	Missense	Unknown
S373P	7	Missense	Unknown
K393N	10	Missense	Unknown
R397W	5	Missense	Unknown
P400fs/62 [ins C 1201-1022]	6	Frameshift	Haploinsufficiency
P448fs/13 [ins G 1344-1345]	11	Frameshift	Haploinsufficiency
I517T	3	Missense	Unknown
R518X [1552 C>T]	11	Nonsense	Haploinsufficiency (q)
M520R	3	Missense	Unknown
V524G	4	Missense	Unknown
Q530X [1588 C>T]	13	Nonsense	Haploinsufficiency (q)
R562 mol/L	2	Missense	Unknown
S566F	3	Missense	Unknown
I567S	6	Missense	Unknown
S571fs/20 [del C 1714]	3	Frameshift	Haploinsufficiency
R591C	5	Missense	Unknown
R591H	6	Missense	Haploinsufficiency (r)
R594Q	11	Missense	Haploinsufficiency (q)
D611Y	10	Missense	Haploinsufficiency (s)
A636fs/28 [del C 1909]	2	Frameshift	Haploinsufficiency
Intron			
IVS2+1 G>A	2	Splice site	Unknown
IVS4+5 G>A	2	Splice site	Unknown
IVS7+5 G>A	15	Splice site	Unknown

*The numbers and letters refer to the amino acid coding of the mutant channel protein. The brackets contain the nucleotide code for deletions, frameshift, splice site, and nonsense mutations.

†Included in this table are 52 subjects who died suddenly at a young age. These subjects were from families with a known KCNQ1 mutation and were assumed to have their respective family mutation.

‡Dominant-negative effect is associated with >50% reduction whereas haploinsufficiency is associated with <50% reduction in ion channel repolarizing current. See text for details. Letters in parentheses refer to references that are available in the online-only Data Supplement.

mutations were identified. A majority of the mutations were localized to the S1 to S6 transmembrane domains (66%), and missense (single amino acid substitutions) accounted for 81% of all the mutations.

The phenotypic characteristics of patients enrolled in each of the 3 registries and by location and type of mutation are presented in Table 2. The clinical characteristics of the patients were similar among the 3 registries except for QTc duration and frequency of β-blocker use. The QTc interval was longer and cardiac events and β-blocker use were more frequent in patients with mutations in the transmembrane than in the C-terminus location. β-Blockers were used less frequently in patients from the Japanese registry than in patients from the other 2 registries. The frequency of first cardiac events was higher in those with than without missense mutations. The clinical characteristics of the 19 subjects possessing intron mutations resembled those with transmembrane and missense mutations.

The QTc interval was significantly longer in the 12 patients with 2 mutations than in those with only single KCNQ1 mutations (570±70 versus 480±60 ms; $P<0.01$). All 12 patients with 2 mutations experienced at least 1 cardiac event.

The cumulative probabilities of first cardiac event by location and type of mutation are presented in Figure 2A and 2B, respectively. Significantly higher event rates were found in subjects with transmembrane than C-terminus mutations and in those with than without missense mutations, with the most rapid increase in event rates occurring during ages 7 to

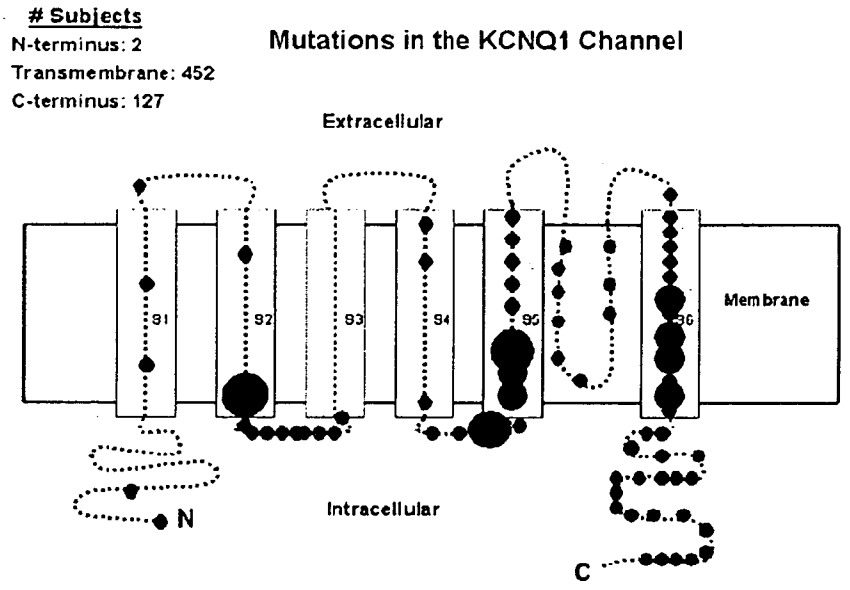

Figure 1. Frequency and location of 74 different mutations in the KCNQ1 potassium channel involving 581 subjects. The 19 subjects with 3 intron mutations are not included in this diagram. The α subunit involves the N-terminus (N), 6 membrane-spanning segments, and the C-terminus portion (C). The size of the circles reflect the number of subjects with mutations at the respective locations, with the small circles indicating <15, medium-sized circles 15 to 30, and large circles >30 subjects.

20 years. In patients with transmembrane-localized mutations, the event rates for patients with mutations localized to the pore region (S5-pore-S6) were nearly identical to those with nonpore mutations (data not shown).

The findings from the Cox regression analysis for location and type of mutation are presented in Table 3. The clinical risk factors associated with first cardiac events involved males before age 13 years, females after age 13

TABLE 2. Phenotypic Characteristics by Source of Subjects, Location of Mutation, and Type of Mutation

	Source of Subjects			Location of Mutation		Missense Mutation		
Characteristics	United States (n=425)	Netherlands (n=93)	Japan (n=82)	Trans Membrane (n=452)	C-Terminus (n=127)	Yes (n=483)	No (n=98)	Intron Mutation (n=19)
Female, %	57	53	54	57	51	54	62	63
ECG at enrollment								
QTc††, ms	488±58	450±45	472±46	485±53	460±61	481±59	471±38	478±60
Therapy, %								
β-Blockers†‡	45	34	26	45	28	42	38	37
Pacemaker	2.4	0	0	1.5	2.4	1.4	3.1	0
Sympathectomy	0.5	0	0	0.4	0	0.4	0	0
Defibrillator	6.4	3.2	0	5.8	3.1	5.2	5.1	0
First cardiac event*‡§, %	41	37	38	45	21	43	26	42
Syncope‡ (n=200)	35	31	29	38	17	36	21	32
Aborted cardiac arrest (n=15)	1.9	1.1	7.3	2.9	0.8	2.5	2.0	5.3
Death (n=23)	4.0	5.5	1.2	4.0	3.1	4.2	2.0	5.3
Ever cardiac event, %								
Syncope‡§	35	31	31	39	17	37	21	33
Aborted cardiac arrest†	2.4	15	8.8	5.3	3.2	5.4	2.0	11
Death	11	14	2.4	10	6.3	11	4.1	26

Plus-minus values are mean±SD. Percentages >10 are rounded to a whole number. The 600 subjects in this table include 51 subjects who died suddenly at a young age, were from families with known KCNQ1 mutation, and were assumed to have the family mutation. Patients with intron mutations are categorized separately and are not included in the location or missense categories. Seven subjects with transmembrane mutations and 1 with C-terminus mutations had missing data about the date of the first cardiac event. Eight subjects with missense mutations had missing data about the date of the first cardiac event. Numbers in parentheses indicate the total number of specific first cardiac events from the 3 sources of patients.

*First cardiac event was syncope, aborted cardiac arrest, or sudden death, whichever occurred first.

†$P<0.01$ for the comparison of characteristics among the 3 sources of subjects.

‡$P<0.01$ for the comparison of characteristics between the 2 locations of the mutations.

§$P<0.01$ for the comparison of characteristics between missense yes and no.

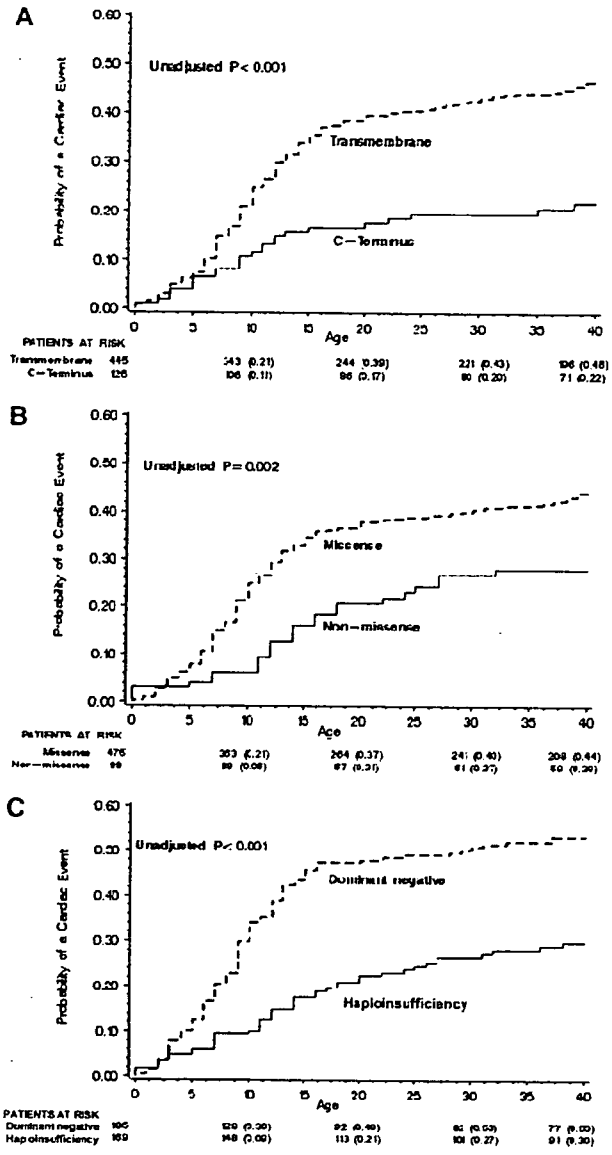

Figure 2. Kaplan-Meier estimate of the cumulative probability of a first cardiac event by location (A), type (B), and biophysical function of the mutation (C).

years, and longer QTc intervals. Mutations located in the transmembrane region of the channel made significant and independent contributions to the risk model, but missense mutations were not an independent risk factor. Three different intron mutations were present in 19 subjects from 4 families, and these intron mutations made a meaningful but nonsignificant contribution to the risk model. Prespecified interactions were investigated for their effect on cardiac events, and no significant interactions were found for transmembrane location by type of mutation, transmembrane location by QTc, or mutation type by QTc. Time-dependent β-blocker use was associated with a significant 74% reduction in the risk of first cardiac events ($P<0.001$).

TABLE 3. Cox Regression With Multiple Predictor Variables Including Location and Type of Mutations for First Cardiac Event

Variable	Hazard Ratio	95% CI	P
Netherlands:United States	1.15	0.74–1.78	0.55
Japan:United States	1.45	0.98–2.16	0.07
Male <13 y:female <13 y	1.72	1.25–2.38	<0.001
Female 13–40 y:male 13–40 y	2.27	1.30–3.96	<0.01
QTc 500–530 ms:QTc <500 ms	2.04	1.41–2.96	<0.001
QTc >530 ms:QTc <500 ms	3.25	2.25–4.69	<0.001
QTc missing*:QTc <500 ms	2.26	1.57–3.25	<0.001
Transmembrane:C-terminus	2.06	1.36–3.12	<0.001
Missense yes:no	1.33	0.86–2.05	0.20
Intron:C-terminus	2.45	0.98–6.11	0.06
Time-dependent β-blocker use	0.26	0.14–0.49	<0.001

The Cox analysis involved 592 subjects with 445 transmembrane, 126 C-terminus, 2 N-terminus, and 19 intron mutations; 8 subjects were not included in this Cox analysis because of missing data about the date of their first cardiac event.

*QTc missing category involves 47 subjects who died suddenly at a young age without a prior ECG.

Biophysical Function and Outcome

The clinical implications of disordered biophysical function of the mutant KCNQ1 channels were investigated in a subset of 356 subjects with known or suspected alteration in ion channel function (see Methods for functional categorization). The clinical characteristics of patients with dominant-negative and haploinsufficiency ion channel dysfunction are presented in Table 4. Patients with mutations having dominant-negative ion current effects had a longer QTc interval and a higher frequency of cardiac events than subjects with mutations resulting in haploinsufficiency. The cumulative probabilities of a first cardiac event by the biophysical function of the mutations are presented in Figure 2C. As shown in Table 5, patients with mutations having

TABLE 4. Phenotypic Characteristics by Biophysical Function of the KCNQ1 Mutations in 356 Subjects

Characteristics	Dominant-Negative Effect (n=187)	Haploinsufficiency (n=169)
Female, %	51	61
ECG at enrollment		
QTc,* ms	500±60	470±50
Therapy, %		
β-Blockers	47	37
Pacemaker	1.1	4.1
Sympathectomy	0.5	0
Defibrillator	4.8	7.7
First cardiac event*, %	53	27
Syncope	45	22
Aborted cardiac arrest	2.1	3.0
Death	5.3	2.4

Percentages >10 are rounded to a whole number. Two subjects had missing data about the date of their first cardiac event.

*$P<0.01$.

TABLE 5. Cox Regression With Multiple Predictor Variables Including Ion Channel Dysfunction for First Cardiac Events

Variable	Hazard Ratio	95% CI	P
Netherlands:United States	2.78	1.48-5.23	<0.01
Japan:United States	1.63	1.02-2.63	0.04
Male <13 y:female <13 y	1.94	1.29-2.91	<0.01
Female 13-40 y:male 13-40 y	1.95	0.99-3.87	0.06
QTc 500–530 ms:QTc <500 ms	1.88	1.18-2.99	<0.01
QTc >530 ms:QTc <500 ms	3.22	2.06-5.05	<0.001
QTc missing*:QTc <500 ms	2.07	1.29-3.33	<0.01
Dominant-negative:haploinsufficiency	2.26	1.56-3.25	<0.001
Time-dependent β-blocker use	0.21	0.09-0.48	<0.001

The analysis involved 354 subjects with known or suspected ion channel dysfunction; 2 subjects were not included because of missing data about the date of their first cardiac event.

*The QTc missing category involves 26 patients who died suddenly at a young age without a prior ECG.

dominant-negative functional effects experienced a significantly greater risk for cardiac events than those with haploinsufficiency (hazard ratio, 2.26; 95% CI, 1.56 to 3.25; $P<0.001$) after adjustment for relevant covariates including QTc and gender effects by age group. β-Blocker use was associated with a significant 79% reduction in first cardiac events in this subset of patients. Because substantial colinearity exists for transmembrane mutations, missense mutations, and mutations with dominant-negative biophysical function, the individual effects of these 3 mutation parameters could not be ascertained reliably in the same Cox model.

Discussion

The main results of the present study from 600 patients having a spectrum of KCNQ1 mutations derived from 3 LQTS registries are significantly higher cardiac event rates in patients with transmembrane mutations and in patients with mutations having a putative dominant-negative effect on the repolarizing I_{Ks} current. The effect of these genetically determined factors is independent of traditional clinical risk factors and of β-blocker therapy.

Since 1995, when the first 2 genes responsible for LQTS were identified,[11,12] molecular genetic studies have revealed a total of 9 forms of congenital LQTS caused by mutations in genes involving potassium channel (LQT-1, -2, -5, -6, and -7), sodium channel (LQT-3, -9), and calcium channel proteins (LQT-8) as well as a membrane-adapter protein (LQT-4).[2,13] Genotype–phenotype studies have enabled us to stratify risk and to treat more specifically patients with LQT-1, LQT-2, and LQT-3 subtypes of this genetic disorder. LQT-1, the most common form of LQTS, accounts for ≈50% of genotyped patients[3,14] and has more variable expressivity and incomplete penetrance than the other forms.[15] Mutation location and knowledge of the functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for LQT-1, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

Mutations in KCNQ1 are responsible for defects in the slowly activating component of the delayed rectifier current I_{Ks}.[16] This current is the main repolarizing current at increased heart rate and is highly sensitive to catecholamines.[3] We speculate that I_{Ks} channels with transmembrane mutations might have reduced responsiveness to the regulatory β-adrenergic signaling of the ion-conduction pathway with more impairment of shortening of the QTc with exercise-related tachycardia than mutations in the C-terminus region.

Functional I_{Ks} channels result from the coassembly of 4 KCNQ1-encoded subunits. A mutated gene encodes a protein with aberrant function, and the presence of both normal and abnormal proteins in the ion channel contributes to a >50% reduction in ion channel function (dominant-negative effect). An alternative mechanism of reduced repolarizing KCNQ1 K^+ current is the inability of mutated subunits to coassemble with normal gene products, such as occurs with a trafficking defect, resulting in a ≤50% reduction in channel function (haploinsufficiency). With only 1 exception,[17] this is the case for all studied truncating mutations leading to incomplete proteins. Our assumption that truncated proteins (based on frameshift nonsense mutations) lead to haploinsufficiency seems justified. The biophysical effect of missense mutations is unpredictable, and both haploinsufficiency and dominant-negative effects have been described. In the absence of reported biophysical studies, missense mutations were classified as unknown.

Previous attempts to identify a genotype–phenotype relationship for KCNQ1 mutations failed to reach consensus on the clinical outcome of the type and site of mutations.[7,8] Relatively small numbers and different ethnic background of the previously reported patients with the LQT-1 genotype might be responsible for the discrepant results. The present larger study allows us to demonstrate for the first time that the biophysical effect clearly affects the clinical outcome (ie, dominant-negative mutations are associated with a more severe phenotype than are mutations conferring haploinsufficiency [Figure 2C], even after adjustment for relevant covariates [Table 5]). The risk observed in 19 subjects with 3 different intron mutations was not quite significant ($P=0.06$), possibly because of small numbers, but the magnitude of the risk effect was similar to the risk accompanying transmembrane mutations. Although these intron mutations produced splice-site alterations predicted to affect the transmembrane portion of the ion channel, we used a separate categorization of intron mutations in view of the limited understanding of the structural alterations and functional effects resulting from these exon-skipping intron mutations.

A few additional findings from this large genotype–phenotype study of type-1 LQTS patients emphasize high risk for first cardiac events during adolescence, a crossover in risk by sex at approximately age 13 years, and a lower rate of first cardiac events in the adult years than in the younger years. These findings are not especially new,[18,19] but the present study highlights their presence in type-1 LQTS.

Study Limitations

The present study used the biophysical function of mutations reported in the literature in only a portion of the mutations

that were included (see references associated with Table 1 in the online-only Data Supplement). The published studies were from many different laboratories with the use of different cellular heterologous expression systems involving *Xenopus* oocytes and other cells at both room and physiological temperatures. Although such nonuniform testing may have contributed to some inconsistency in the categorized biophysical function, the finding of a significantly higher event rate in mutations with dominant-negative than with haploinsufficient effects (hazard ratio, 2.26; $P<0.001$) is unlikely to have resulted from the nonuniform testing. Unfortunately, we did not have the resources to perform such uniform testing in all 77 mutations presented in the present study.

Once a mutation was identified in KCNQ1, thorough genetic sequencing was not performed routinely in all the ion channel genes to look for second mutations. Thus, some of the patients included in the analysis may have had a second mutation in addition to the identified KCNQ1 mutation. It is estimated that ≈10% of genotype LQTS patients may carry a second mutation, and those with >1 mutation could contribute to some of the findings in our study. In addition, it is possible that some of the reported mutations (Table 1) are simply uncommon sequence mutations, but this is relatively unlikely because all the subjects in the present study were derived from families in which the proband had QTc prolongation not due to a known cause.

The outcome analyses included subjects from families with a known KCNQ1 mutation who died suddenly and unexpectedly at a young age and were classified as LQTS-related death with the same mutation that was present in the family. It is possible that a few of these subjects could have died from a non-LQTS cause or had an LQTS mutation different from the family mutation, but we think the error rate is likely to be small. The number of deaths and aborted cardiac arrest events is small, and there is insufficient power to evaluate the risk association of the genotype characteristics with these endpoint events in a multivariate time-dependent model.

Conclusions

The present study confirms that in patients with type-1 LQTS, longer QTc intervals are associated with higher cardiac event rates and that male patients are generally younger than female patients at first cardiac events.[20,21] The new findings from the present study are that transmembrane mutations and mutations with dominant-negative functional effect adversely influence the outcome of this disorder independent of traditional clinical risk factors and β-blocker therapy. The present study was not designed to assess the effectiveness of different therapies in patients with KCNQ1 mutations. The findings presented do not provide justification for using specific genotype characteristics to identify patients for implanted defibrillator therapy.

Note Added in Proof

After this article was accepted for publication, we noted the recent article by Tsuji et al, in which the A344A/sp [1032G>A] mutation that we categorized as haploinsufficient (Table 1) was reported to have a weak dominant-negative effect.[22] We reran the KCNQ1 data recategorizing the 27 A344A/sp [1032 G>A] mutations as dominant-negative. Negligible changes occurred in the results as presented in Table 5 and Figure 2C; the hazard ratio for dominant-negative:haploinsufficiency (Table 5) was unchanged at 2.26 ($P<0.001$).

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Disclosures

Dr Ackerman is a consultant for Clinical Data (formerly Genaissance Pharmaceuticals) with respect to the FAMILION genetic test for cardiac ion channel mutations. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Type-1 long-QT syndrome is caused by loss-of-function mutations in the KCNQ1-encoded I_{Ks} cardiac potassium channel. In the present study involving 600 patients having a spectrum of KCNQ1 mutations derived from 3 long-QT syndrome registries, we found that cardiac event rates are increased significantly in patients with mutations located in the transmembrane region of the potassium channel and in patients with mutations having a putative dominant-negative effect on the repolarizing I_{Ks} current. The effects of these genetically determined factors are independent of traditional clinical risk factors and of β-blocker therapy. Mutation location and knowledge of functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for type-1 long-QT syndrome, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

The endothelial nitric oxide synthase gene -786T/C polymorphism is a predictive factor for reattacks of coronary spasm

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Objective We previously found a -786T/C polymorphism in the 5′-flanking region of the endothelial nitric oxide synthase (eNOS) gene and reported that this polymorphism is strongly associated with coronary spasm. In this study, we examined whether the polymorphism is a prognostic marker in coronary spasm patients.

Methods and results We examined the clinical courses of 201 consecutive patients with coronary spasm who were admitted to our institution: 146 patients with the -786T/T genotype; 50 patients with the -786C/T genotype; and five patients with the -786C/C genotype. The mean follow-up period was 76±60 months. All the patients took calcium channel blockers and/or nitrate during the follow-up period. In this study, no patients died due to a cardiac event. About 25 patients were readmitted owing to cardiovascular disease. Out of these 25 patients, 23 patients were readmitted owing to a reattack of coronary spasm. The -786C allele was significantly associated with readmission due to coronary spasm ($P=0.0072$, odds ratio: 3.37 in the dominant effect). Kaplan–Meier analysis revealed that the occurrence of readmission was significantly higher in the patients with the -786C allele than in the patients without the -786C allele ($P=0.0079$). Further, multiple logistic regression analysis revealed that the -786T/C polymorphism was an independent predictor for readmission due to reattack of coronary spasm ($P=0.006$; relative risk=3.590).

Conclusions The eNOS -786C allele is an independent risk factor for readmission due to a recurrent attack of coronary spasm in patients with coronary spasm, even if the patients have taken calcium channel blockers and/or nitrate. ***Pharmacogenetics and Genomics*** 17:581–587 © 2007 Lippincott Williams & Wilkins.

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Introduction

Coronary spasm plays an important role in the pathogenesis of not only variant angina but also ischemic heart diseases in general, including other forms of angina pectoris, acute myocardial infarction, and sudden death [1–3].

We previously reported that the long-term prognosis for patients with coronary spasm is relatively good and that the use of calcium channel blockers (CCBs) improves it [4,5].

We have recommended that patients with coronary spasm take CCB, which dilates the large coronary arteries and thereby prevents the occurrence of coronary spasm; however, there were many patients who were readmitted owing to a recurrent attack of coronary spasm while on the CCB regimen [6]. It is not yet clear what factor(s) predisposes coronary-spasm patients who take CCB to a reattack of coronary spasm.

We previously reported that a -786T/C polymorphism in the 5′-flanking region of the endothelial nitric oxide synthase (eNOS) gene is strongly associated with coronary spasm; it also results in a significant reduction in eNOS gene promoter activity [7]. We further showed that the replication protein A1 binds to the -786C allele and thereby represses eNOS gene transcription [8]. Our clinical research revealed that the polymorphism strongly increases the basal tone of the coronary arteries, and enhances their response to the constrictor effects of acetylcholine (ACh) [9,10]; furthermore, we reported

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that the polymorphism is significantly associated with myocardial infarction, especially in patients without coronary stenosis [11]. These findings suggest that the -786T/C polymorphism in the eNOS gene compromises endothelial NO synthesis and thereby predisposes the patients to severe coronary spasm.

In this study, we examined the association between the -786T/C polymorphism and the long-term prognosis for coronary-spasm patients.

Methods

Study population

The study population consisted of 201 consecutively admitted patients from July 1984 to July 2000 (100 men; 101 women: mean age in years, 61.9; range 25–81 years) from whom genomic DNA could be obtained. All of them had coronary spasm as defined by an intracoronary injection of ACh. Coronary spasm was defined as total or subtotal occlusion of a coronary artery, which was associated with ischemic electrocardiographic changes and/or chest pain. Patients with significant organic stenosis in the coronary arteries, defined as having more than 50% organic stenosis in at least one coronary artery after nitroglycerine administration, were excluded. Patients who stopped medications were excluded from this study.

The patients were divided into two groups: the -786T group consisting of 146 patients with the -786T/T genotype (74 men and 72 women; mean age in years, 61.7; range, 25–81) and the -786C group consisting of 55 patients (28 men and 27 women; mean age in years, 62.2; range, 40–77). The latter group included 50 patients with the -786C/T genotype and five patients with the -786C/C genotype. We examined the following two events during the follow-up period: (i) death from all causes and (ii) readmission due to a coronary arterial event, such as reattack of coronary spastic angina, angina pectoris due to organic stenosis, or acute myocardial infarction.

Cardiac catheterization

All medications taken by the study participants were discontinued at least 48 h before cardiac catheterization. Coronary arteriography was performed in the morning while the patients were in a fasting state. After baseline arteriography of the left and right coronary arteries, an intracoronary injection of ACh was administered as described previously [12–15].

Two consecutive doses (50 and 100 μg) of ACh were administered 4 min apart, injected into the left coronary artery: angiography was performed within 30 s of each injection. Then, 50 μg of ACh was injected into the right coronary artery and angiography was again performed. Finally, both left and right coronary arteriograms were taken after an intracoronary injection of 1 mg of isosorbide dinitrate. We evaluated the degree of organic stenosis after the injection of isosorbide dinitrate.

Screening method for the -786T/C polymorphism in the endothelial nitric oxide synthase gene

An allele-specific oligonucleotide method was used in the screening for the -786T/C polymorphism in the eNOS gene. Hybridization was accomplished with ^{32}P-radiolabeled oligonucleotides corresponding to either the probe for the -786T allele or the probe for the -786C allele. The method has been described earlier. [7]. In brief, the PCR fragments, 236-bp in length, including the -786T/C polymorphism site, were blotted in duplicate onto nylon membranes. Hybridization was accomplished with ^{32}P-radiolabeled oligonucleotides corresponding to either the -786T sequence (5′-GGG TCA GCC AGC CAG GGAA-3′; probe for the -786T sequence) or the -786C sequence (5′-GGG TCA GCCGGC CAG GGAA-3′; probe for the -786C sequence).

Statistical analysis

Continuous variables are expressed as mean ± SD. Mean values were compared using the unpaired Student's *t*-test. The χ^2 test was used for the evaluation of differences between proportions. A probability value < 0.05 was considered to indicate statistical significance.

Multiple logistic regression analysis with forward stepwise selection was performed with SPSS 14.0J for Windows (SPSS Japan Inc). Multiple logistic analysis was used to determine independent predictors of coronary spasms. Independent variables were coded as the following dummy variables: genotype, 0 for the -786T/T genotype and 1 for the -786C/T or the -786C/C genotype; sex, 0 for women and 1 for men; age, 0 for < 55 years and 1 for ≥ 55 years; body mass index, 0 for $< 25\,kg/m^2$ and 1 for $\geq 25\,kg/m^2$; hypercholesterolemia, 0 for < 220 mg/dl and 1 for ≥ 220 mg/dl; Cigarette smoking, 0 for nonsmokers and 1 for ex-smokers (all study participants quit smoking upon admission); hypertension, 0 for normotension and 1 for hypertension; and diabetes mellitus, 0 for an absence and 1 for a presence. A Kaplan–Meier survival curve was used for determining survival and readmission rates in both the -786T group and the -786C group. We compared the survival rates and readmission rates between the -786T group and the -786C group using the log-rank test.

Results

Follow-up periods

The patients in this study were followed up until 1 December 2005. The mean follow-up period was 76 ± 60 months (range 1–252 months) for all study patients, with mean follow-up periods of 74 ± 56 months (range 1–252 months) for the -786T group and 81 ± 68 months (range 1–235 months) for the -786C group.

Clinical characteristics in the study patients

The clinical characteristics of the study patients are shown in Table 1. The incidence of coronary risk factors, including age, sex, hypertension, and cigarette smoking, were compared between the -786T and the -786C groups. No significant differences were seen in these coronary risk factors between the -786T and the -786C groups. No significant differences were seen in the drug regimens, including CCB, nitrates, angiotensin-converting enzyme inhibitors (ACE-I), or antiplatelets between the two groups.

Prognosis of patients and causes of death

In this study population, 192 survived and nine died during the follow-up period. Of the nine patients who died, three died of lung cancer, one of pancreas cancer, one of a brain tumor, one of a ruptured thoracic aortic aneurysm, one of stroke, and two of respiratory failure. Kaplan–Meier analysis revealed that there were no significant differences in the death rates between the -786T group and the -786C group (log-rank test: $P = 0.5945$) (Fig. 1).

Readmission due to coronary arterial disease and the -786T/C polymorphism

Twenty-five patients were readmitted owing to a recurrence of coronary arterial disease. Out of these 25 patients, 23 patients were readmitted owing to a reattack of coronary spasm. In the patients readmitted owing to a reattack of coronary spasm, one patient was readmitted owing to acute myocardial infarction without significant organic stenosis. He had the -786C/C genotype of the eNOS gene. Two patients who were readmitted owing to a progression of coronary stenosis had the -786T/T genotypes.

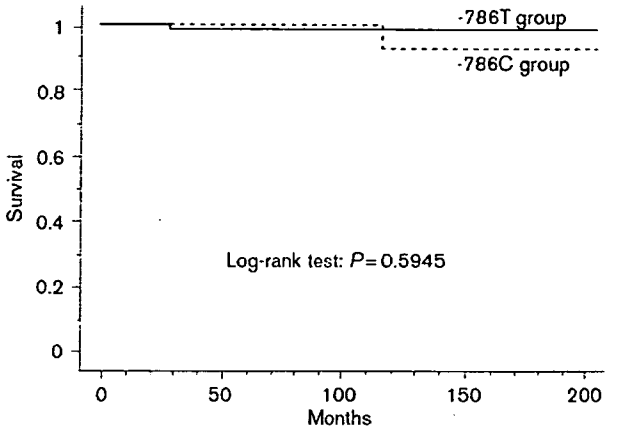
The rate of readmission due to coronary arterial disease was significantly higher in the -786C group than in the -786T group ($P = 0.0134$) [21.8% (12/55) and 8.9% (13/146), respectively, as is shown in Table 2]. The rate of readmission due to a reattack of coronary spasm was significantly higher in the -786C group than in the -786T group ($P = 0.0046$) [21.8% (12/55) and 7.5% (11/146), respectively].

Table 1 **Clinical characteristics of the study patients**

	-786T group ($n = 146$)	-786C group ($n = 55$)	*P* value
Age (years)	62 ± 11	62 ± 10	NS
Men/women	72/74	28/27	NS
Hypertension	44/146 (30%)	15/55 (27%)	NS
Cigarette smoking	78/146 (53%)	33/55 (60%)	NS
Diabetes mellitus	28/146 (19%)	11/55 (20%)	NS
Hypercholesterolemia	41/146 (28%)	13/55 (24%)	NS
BMI (kg/m^2)	23 ± 3	23 ± 3	NS
Pharmacotherapy			
CCB	137/146 (94%)	52/55 (95%)	NS
Nitrates	20/146 (14%)	4/55 (7%)	NS
ACE-I	12/146 (8%)	7/55 (13%)	NS
Antiplatelet	16/146 (11%)	6/55 (11%)	NS
HMG-CoA Reductase inhibitor	18/146 (12%)	2/55 (4%)	NS

Values are numbers of patients or mean ± SD.
ACE-I, angiotensin-converting enzyme inhibitor; BMI, body mass index; CCB, calcium channel blocker; NS, not significant.

Fig. 1



Kaplan–Meier survival curves of cumulative death rates in patients with coronary spasm divided into two groups according to the -786T/C polymorphism.

Table 2 **Readmission rates**

	-786T group ($n = 55$)	-786C group ($n = 146$)	*P* value
Reattack of coronary spasm	11/146 (7.5%)	12/55 (21.8%)	0.0046
Progression of coronary stenosis	2/146 (1.4%)	0/55 (0%)	0.3830
Total	13/146 (8.9%)	12/55 (21.8%)	0.0134

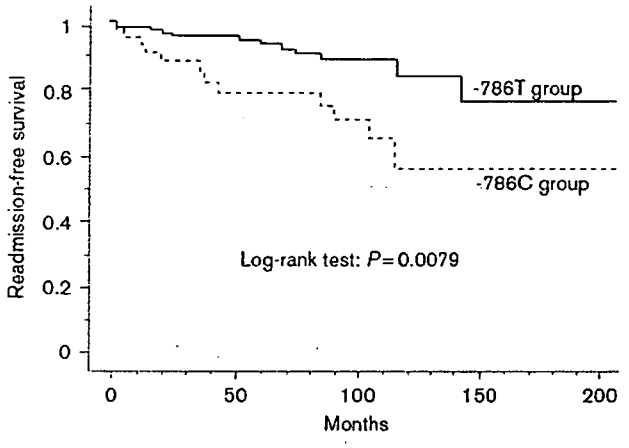
Values are numbers of patients.

Kaplan–Meier analysis revealed that the occurrence of readmission due to coronary arterial disease was significantly lower in the -786T group than in the -786C group ($P = 0.0079$) (Fig. 2). Further, the occurrence of readmission due to coronary spasm was significantly lower in the -786T group than in the -786C group ($P = 0.0032$) (Fig. 3).

Risk factors for readmission due to a reattack of coronary spasm

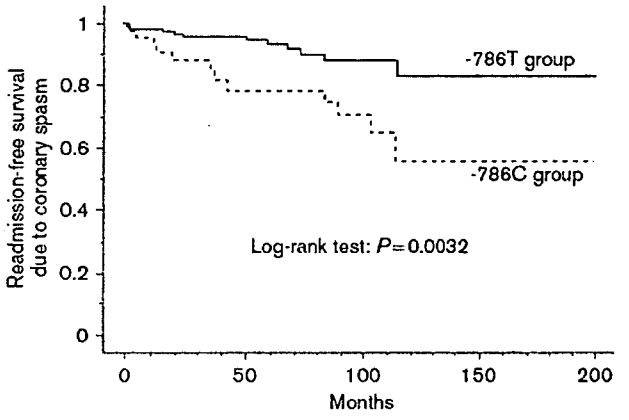
We compared the coronary risk factors, including clinical coronary risk factors and medications, between the readmission and the non-readmission groups as is shown in Table 3. Patients in the group readmission due to a reattack of coronary spasm were significantly younger than those in the non-readmission group ($P = 0.0044$). No significant differences were seen in the other coronary risk factors or in the medications between the readmission and the non-readmission groups.

Fig. 2



Kaplan–Meier survival curves of cumulative readmission rates in patients with coronary spasm divided into two groups according to the -786T/C polymorphism.

Fig. 3



Kaplan–Meier survival curves of cumulative readmission rates due to coronary spasm in patients with coronary spasm divided into two groups according to the -786T/C polymorphism.

The rate of readmission due to a reattack of coronary spasm in the patients with T/T, C/T, and C/C genotypes were 7.6% (11/144), 22.0% (11/50), and 20.0% (1/5), respectively. The incidence of patients with the -786T/C polymorphism was significantly higher in the group of the readmission due to a reattack of coronary spasm than in the non-readmission group ($P = 0.0051$). When the additive and dominant effect of the -786C allele was analyzed, the -786C allele was significantly associated with readmission due to coronary spasm as is shown in Table 4 ($P = 0.0072$, odds ratio: 3.37 in the dominant effect).

Table 3 Clinical characteristics in readmission due to coronary spasm and non-readmission groups

	Non-readmission ($n=176$)	Readmission ($n=23$)	P value
Age (years)	63±10	56±12	0.0044
Men/women	86/90	12/11	NS
Hypertension	51/176 (29%)	8/23 (35%)	NS
Cigarette smoking	95/176 (54%)	14/23 (61%)	NS
Diabetes mellitus	37/176 (21%)	2/23 (9%)	NS
Hypercholesterolemia	47/176 (27%)	7/23 (30%)	NS
BMI (kg/m^2)	23±3	23±3	NS
Pharmacotherapy			NS
CCB	167/176 (95%)	22/23 (96%)	NS
Nitrates	20/176 (11%)	3/23 (13%)	NS
ACE-I	16/176 (9%)	2/23 (9%)	NS
Antiplatelets	22/176 (13%)	0/23 (0%)	NS
HMG-CoA Reductase inhibitor	16/176 (9%)	2/23 (9%)	NS

Values are numbers of patients or mean ± SD.
ACE-I, angiotensin-converting enzyme inhibitor; BMI, body mass index; CCB, calcium channel blocker; NS, not significant.

Table 4 Genotype frequencies of -786T/C polymorphism in the readmission due to coronary spasm and non-readmission group

	Non-readmission ($n=176$)	Readmission ($n=23$)	Odds ratio (95% CI)	P value
-786C/C genotype	4/176 (2%)	1/23 (4%)	-	-
	24%	52%		
-786C/T genotype	39/176 (22%)	11/23 (48%)		
-786T/T genotype	133/176 (76%)	11/23 (48%)	-	-
Additive	-	-	2.56 (1.26–5.21)	0.0097
Dominant	-	-	3.37 (1.39–8.20)	0.0072
Recessive	-	-	1.96 (0.21–18.29)	0.5569

Values are numbers of patients. CI, confidence interval.

Subsequently, we performed multiple logistic analysis, with forward stepwise selection for the readmission group, using all the clinical risk factors and the -786T/C polymorphism as shown in Table 5. The analysis revealed that the most predictive independent risk factor for readmission due to a reattack of coronary spasm was the -786T/C polymorphism ($P = 0.006$, relative risk = 3.590). Other classical coronary risk factors were not significant predictive factors for readmission due to coronary spasm.

CCB and readmission due to coronary spasm

We analyzed compounds and doses of CCBs, which were administered to patients with coronary spasm as shown in Table 6. The incidence of readmission due to coronary spasm was significantly higher in patients who were administered two compounds of CCBs than in patients who were administered one compound of CCBs ($P = 0.0016$).

We listed nine patients who were administered two compounds of CCBs as shown in Table 7. The incidence of the -786T/C polymorphism in the readmission and non-readmission groups were 4/4(100%) and 1/5(20.0%),

Table 5 Multiple logistic analysis with forward stepwise selection for readmission due to coronary spasm

Variables	β	SE	Relative risk (95% CI)	*P* value
-786T/C Polymorphism	1.278	0.461	3.590 (1.455–8.853)	0.006
Age	−0.931	0.489	0.432 (0.151–1.028)	0.057
Constant	−2.141	0.346	0.117	0.000

CI, confidence interval.

Table 6 Compounds and doses of CCBs in the readmission due to coronary spasm and the non-readmission group

Compounds and doses (dose per day)	Readmission (n=23)	Non-readmission (n=176)	*P* value
Diltiazem (long acting type)			
200 mg	5/23 (22%)	67/176 (38%)	NS
100 mg	3/23 (13%)	32/176 (18%)	NS
Diltiazem (short acting type)			
240 mg	0/23 (0%)	2/176 (1.2%)	NS
180 mg	1/23 (4%)	3/176 (2%)	NS
150 mg	0/23 (0%)	1/176 (0.6%)	NS
120 mg	2/23 (8%)	8/176 (5%)	NS
90 mg	0/23 (0%)	5/176 (3%)	NS
60 mg	0/23 (0%)	1/176 (0.6%)	NS
Nisoldipine			
20 mg	1/23 (4%)	3/176 (1.7%)	NS
15 mg	1/23 (4%)	0/176 (0%)	NS
10 mg	4/23 (17%)	21/176 (12%)	NS
5 mg	0/23 (0%)	6/176 (3%)	NS
Nifedipine			
80 mg	1/23 (4%)	1/176 (0.6%)	NS
60 mg	0/23 (0%)	1/176 (0.6%)	NS
40 mg	0/23 (0%)	2/176 (1.2%)	NS
20 mg	0/23 (0%)	4/176 (2.2%)	NS
Benidipine			
8 mg	0/23 (0%)	2/176 (1.2%)	NS
Amlodipine			
5 mg	0/23 (0%)	2/176 (1%)	NS
2.5 mg	0/23 (0%)	1/176 (0.6%)	NS
Two CCB compounds	4/23 (17%)	5/176 (3%)	0.0016

Values are numbers of patients.
CCB, calcium channel blocker; NS, not significant.

respectively. In the patients with two compounds of CCBs, the incidence of the -786T/C polymorphism was significantly higher in the readmission group than in the non-readmission group ($P = 0.0164$).

Discussion

Prognosis and readmission in patients with coronary spasm

As with others, we have previously reported that the prognosis for coronary-spasm patients without coronary stenosis was relatively good [4,5]. We have also reported that the intake of CCB, multivessel spasm, and the severity of coronary artery disease are all significant independent predictors of survival for patients without myocardial infarction [4]. In this study population, patients with significant organic stenosis were excluded. All the study patients took CCB and/or nitrate during the follow-up period; there were no cardiac deaths during this period in this study. This result is in accordance with many previous studies. On the other hand, there were 25 study patients (12%) who were readmitted owing to coronary events: 92% of the readmissions were due to a reattack of coronary spasm. Sueda *et al.* [6] recently suggested that 42% of the patients with pure coronary spastic angina had a reattack of coronary spastic angina during the administration of CCB. The outcomes in Sueda's coronary-spasm patients are in general agreement with our results.

Table 7 Characteristics of patients who were administered CCB two compounds

Patient no.	Age	Sex	-786T/C genotype	Hypertension	Cigarette smoking	Diabetes mellitus	Hypercholesterolemia
Non-readmission							
1	53	M	T/T	−	+	−	−
2	64	M	T/T	+	+	−	−
3	66	F	T/T	+	+	−	−
4	68	M	T/T	−	+	−	−
5	74	F	C/T	−	−	−	−
Readmission							
6	40	M	C/T	−	+	−	+
7	66	M	C/T	+	−	−	+
8	67	M	C/C	−	+	−	−
9	76	F	C/T	−	−	−	−

F, female; M, male.

In this study, the readmitted patients were significantly younger than the non-readmitted patients. We therefore suggest that the disease-activity level of coronary spastic angina is higher in younger patients than in the older ones. Younger patients may be more susceptible to getting coronary spastic angina as a result of coronary spasm. Further study is needed to clarify whether there is a significant difference in the disease activity level of coronary spasm between younger and older patients.

Readmission and the endothelial nitric oxide synthase polymorphism

The incidence of readmission was significantly higher in the -786C group than in the -786T group. The -786T/C polymorphism was significantly associated with readmission due to coronary arterial events. Multiple logistic regression analysis revealed that the -786C allele was the most predictive independent risk factor for readmission in patients with coronary spasm. It is possible that the -786T/C polymorphism reduces eNOS gene expression in the coronary arterial endothelial cells, and thereby predisposes the patients to recurrent coronary spasm even if the patients have taken CCB.

Readmission due to acute myocardial infarction

In this study, there was a patient who had acute myocardial infarction during the follow-up period. Significantly, he had the -786C/C genotype. As this patient had no coronary stenosis, myocardial infarction was most probably caused by coronary spasm. Although this patient had taken CCB, he had an incident of acute myocardial infarction. Thus, it is suggested that the -786T/C polymorphism predisposes patients to have myocardial infarction due to coronary spasm, even while being administered CCB.

Treatment of coronary spasm in patients with the -786C allele

Strict follow-up is necessary in coronary spasm patients with the -786C allele to monitor for reattack and/or acute myocardial infarction. Additional medications like long-acting CCB and/or nitrite and/or other antiangina agents, should be prescribed for coronary-spasm patients with the -786C allele; however, there was no significant difference with the readmission rates between those with long-acting CCB and those with short-acting CCB in this study.

It was revealed that the incidence of readmission due to a reattack of coronary spasm was significantly higher in patients who were administered two CCB compounds than in patients who were administered one CCB compound; moreover, all the readmission patients with two CCB compounds carried the -786C allele. We usually medicate a patient who has severe and/or medicine-resistant coronary spasm, with a combination of two CCB compounds. These results indicate that a combination of two CCBs is effective in patients without the -786T/C polymorphism, but is not effective in the severe coronary-spasm patients with the -786T/C polymorphism. Additional medications such as HMG-CoA reductase inhibitor, ACE-I, or angiotensin II type 1 receptor blocker are possibly needed in the patients with severe coronary spasm with the -786T/C polymorphism.

We recently reported that fluvastatin, an HMG-CoA reductase inhibitor, increases the transcriptional activity of the eNOS gene in the endothelial cells, especially in those with the -786C allele [16]. We therefore suggested that fluvastatin possibly prevents reattacks of coronary spasm, especially in patients with the -786C allele. It was reported that ACE-I or angiotensin II type 1 receptor blocker induces eNOS bioactivity; therefore, those drugs possibly are effective in the patients with coronary spasm [17,18]. A further clinical study is, however, necessary to verify this.

Study limitation

It has been previously suggested that the pathogenesis of coronary-artery spasm is closely related to the process of atherosclerosis [19,20]; however, in this study, there were relatively few patients who were readmitted owing to a progression of coronary stenosis. A longer follow-up period might be necessary to elucidate whether the incidence of patients with coronary stenosis will increase. Also, there may be racial, and/or environmental, and/or lipid-profile differences in the pathogenesis of atherosclerosis. A study on a larger follow-up population will be beneficial to further elucidate this topic.

The -786T/C polymorphism has only a modulatory role in the development and the recurrence of coronary spasms, which possibly also occur in some patients with angiographically detectable stenoses; therefore, further study will be necessary to elucidate other predictive factors for coronary spasm.

Conclusion

Considering the strong association of the C allele of the eNOS gene -786T/C polymorphism with the prognosis for coronary spastic angina patients, we conclude that the -786T/C polymorphism is an independent predictor for readmission in patients with coronary spasm. The -786T/C polymorphism of the eNOS gene is an important factor to consider in determining the clinical course of coronary spastic angina. A strict follow-up is necessary in coronary-spasm patients with the -786C allele. There is no simple test to measure for the -786T/C polymorphism at present; however, if a test is developed in the near future, it will be valuable for treating patients with coronary spasm, especially those with -786T/C polymorphism.

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