Variable Expression of Long QT Syndrome Among Gene Carriers from Families with Five Different HERG Mutations

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Objectives: This study assessed the phenotypic variability of LQTS in carriers with the same and with different mutations in the LQT2 gene.

Background: Mutations of ion-channel genes are known to cause the long QT syndrome (LQTS), a disorder associated with distinctive genotypic-specific electrocardiographic patterns and variable clinical expression.

Methods: Clinical and electrocardiographic characteristics were assessed in five large LQTS families, each with a different mutation of the HERG gene (LQT2; n = 469, 69% genotyped, 102 carriers). One mutation was located on the N-terminus and the other four on the C-terminus of the HERG channel protein.

Results: The QTc duration and the frequency of cardiac events (syncope and LQTS-related cardiac arrest/death) were similar among carriers with the five HERG mutations. QTc was as variable in carriers of the same mutation as it was among carriers with different HERG mutations (P = 0.19). Qualitative assessment of the electrocardiograms revealed extensive intra- and interfamilial variability in T-wave morphology. Among carriers with multiple electrocardiograms extending over 2 to 7 years, variation in QTc over time was minimal. A strong association was found between QTc and the occurrence of cardiac events in carriers of all five mutations.

Conclusions: The clinical expression of LQTS was equally variable in carriers from families with the same or different HERG mutations. These findings highlight the complexity of the clinical phenotype in this Mendelian dominant disorder and suggest that one or more modifier genes contribute to the variable expression of this syndrome.

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The hereditary long QT syndrome (LQTS) is a familial disorder characterized by prolonged ventricular repolarization and a propensity for syncope and arrhythmic sudden death.¹⁻³ Multiple mutations in several ion-channel genes (KVLQT1, HERG, SCN5A, KCNE1, and KCNE2) have been shown to cause this syndrome.⁴⁻⁸ Various phenotypic characteristics that include electrocardiographic (ECG) patterns,⁹ triggers of cardiac events,^{10,11} and clinical outcome¹² have been reported to be gene-specific. This study was designed to assess the variable phenotypic expression of LQTS within and between families with different mutations in the same LQTS gene as a means of gaining insight into the possible presence of modifier genes in this Mendelian dominant disorder.

METHODS

Study Population

The study population consists of five large LQTS families, with each family having a different HERG mutation by genetic analysis. The family selection criteria required at least 25 members per family with a minimum of 10 members per family with gene mutations. Nongenotyped family members who had a parent or sibling and a child with gene mutations were categorized as obligate carriers. The five different HERG mutations were identified using standard genetic tests.¹³⁻¹⁴ All subjects or their guardians provided informed consent for the genetic and clinical studies.

Phenotypic Characterization

Routine clinical and ECG parameters were acquired at the time of clinical diagnosis. Measured ECG parameters included QT, QT_{peak}, QT_c, and RR intervals in milliseconds.⁹ Repolarization measurements were made manually in three leads (II, V₂, and V₆) with quantitative QT values reported for lead II since the measurements were similar in all three leads. Genotype-phenotype relationships were assessed by QT_c distributions and by a QT_c categorization scheme adjusted for age and gender¹⁵ among genetically tested family members. Cardiac events included syncope, aborted cardiac arrest, and LQTS-related death (sudden unexpected death before 41 years of age, without a known cause).

Statistical Analysis

Differences in clinical characteristics among the five mutation carrier groups were evaluated by analysis of variance. Characteristics of mutation carriers with and without outcome events were evaluated using the Chi-square test. Cumulative probabilities of first cardiac events were assessed by the Kaplan-Meier method,¹⁶ and the results compared with the log-rank test.

RESULTS

Families

The five families included 469 family members with 394 (86%) having complete follow-up information. Genotyping was carried out in 323 family members: 102 (32%) were carriers of HERG mutations, and 87 of the 102 carriers had a recorded ECG; 221 (68%) were noncarriers, and 193 of the 221 noncarriers had ECGs. The preponderance of noncarriers in the five families reflects the fact that more genotyping was requested in family members with nondiagnostic than diagnostic QT_c prolongation values on the ECG.

HERG Mutations

Five different HERG mutations were identified in the five families. Table 1 depicts the location, mechanism, and functional effects of the specific mutations affecting the I_{Kr} current.

Clinical Characteristics by Mutation

Clinical characteristics by HERG mutation among carriers are presented in Table 2. The clinical characteristics and the ECG findings were similar among the five carrier subgroups. Qualitative assessment of ECGs by mutation revealed substantial intra- and interfamilial variability in morphological features (Fig. 1). Among 37 carriers who had multiple tracings over 2 to 7 years, there was an overall 4% reduction in the QT_c interval during a mean follow-up of 68 months. The QT_c changes over time were similar among subjects with the five mutations (Table 3).

Phenotype/Genotype Relationships

Phenotypic characterization of genotyped family members with ECGs was carried out with QT_c categorization of the study population into affected, unaffected, and equivocal (borderline)

Table 1. Location, Functional Effects, and Mechanism of Channel Dysfunction of Five HERG Mutations

HERG Mutation	Site	I _{Kr} Current *	Mechanism	Reference
M124R (missense)	N-terminus; PAS domain	Reduced	Accelerated deactivation to closed channel state	Kass R, Attali B (personal communication) 17
R752W (missense)	Linker C-terminus	Absent	Trafficking defect with mutant channel protein retained in endoplasmic reticulum and not delivered to the plasma membrane	
F805C (missense)	C-terminus	Absent	Trafficking defect similar to R752W	Brown A, Ficker E (personal communication) 18
V822M (missense)	C-terminus	Absent	Trafficking defect with mutant channel protein retained in endoplasmic reticulum and not delivered to the plasma membrane	
W1001Stop (nonsense)	C-terminus	Reduced	Trafficking defect with reduced co-assembly of channel protein	Brown A, Ficker E (personal communication)

* I_{Kr} current as determined in homozygous cellular expression studies.

groups. The performance of this categorization among 280 genotyped family members with ECGs (193 noncarriers, and 87 carriers) by mutation is presented in Table 4. In analysis utilizing only subjects categorized as affected or unaffected by QTc criteria, the sensitivity, specificity, and the positive

and negative predictive accuracies of the QTc criteria were quite high. Twenty-three percent of family members had equivocal QTc values, and a third of this equivocal group had LQT2 mutations.

Table 5 summarizes the intra- and intermutation variability in LQT2 phenotype, with the findings

Table 2. Clinical Characteristics in Carriers by Mutation

Characteristic	HERG Mutation					
	M124R (n = 17)	R752W (n = 29)	F805C (n = 11)	V822M (n = 25)	W1001stop (n = 20)	All (n = 102)
Male (%)	59	48	45	52	50	51
Age at 1 st ECG, yrs	34 ± 17	32 ± 21	34 ± 20	25 ± 23	34 ± 23	27 ± 22
Any cardiac event (%)	53	21	38	12	40	29
Age at 1 st event, yrs	18 ± 6	18 ± 10	17 ± 6	9 ± 6	18 ± 10	17 ± 8
Arousal events (%)	25	17	0	33	38	25
Non-arousal events (%)	63	17	67	67	25	43
Cardiac events ≥ 2 (%)	43	4	25	8	21	16
ACA/SCD (%)	20	0	13	4	0	5
Beta blockers, at < 41 yrs (%)	33	25	63	36	35	34
Pacemaker (%)	27	0	0	0	10	6
ECG parameters (mean ± SD)						
RR, msec	861 ± 175	868 ± 185	853 ± 236	820 ± 284	863 ± 177	853 ± 210
QTpeak, msec	371 ± 89	330 ± 52	339 ± 50	326 ± 73	351 ± 58	340 ± 66
QT, msec	466 ± 104	431 ± 53	446 ± 67	427 ± 93	458 ± 68	442 ± 77
QTc, msec	502 ± 77	467 ± 28	487 ± 34	477 ± 53	497 ± 58	483 ± 52

Values are percent. Plus-minus values are mean ± SD. For all between mutation comparisons P = ns by analysis of variance. ACA = aborted cardiac arrest; SCD = sudden cardiac death.

Figure 1. Electrocardiographic T-wave patterns in lead V₆ in two affected family members from each of five families with different HERG mutations. The sex (M/F) and age in years of the two LQTS carriers (#1 and #2) by HERG mutation are M124R (#1:M-25, #2:M-19); R752W (#1:M-39, #2:M-36); F805C (#1:F-58, #2:F-56); V822M (#1:M-19, #2:M-18); and W1001X (#1:F-16, #2:F-16). Different T-wave morphologies and QTc intervals are present in carriers with the same mutation in each family. Paper speed is 25mm/sec.

graphically displayed in Figure 2A. The cardiac events among gene carriers included syncope, aborted cardiac arrest, or LQTS-related death, and the rates were 0% for those with QTc < 440 ms, 32% for those with QTc 440-550 ms, and 60% for carriers with QTc > 550 sec. The QTc distribution and the frequency of cardiac events among noncarriers (only syncope; no aborted cardiac arrest or sudden death occurred in this group) are presented in Figure 2B. There were 23 noncarriers who had syncope (12%), with 17 of the 23 from one family. A considerable overlap exists in the QTc distribution between carriers and non-carriers (Figs. 2A and 2B).

Outcome Events Among Carriers with HERG Mutations

The cumulative probability of a first cardiac event by age 40 among carriers of each of the five mutations is presented in Figure 3. The probability

of experiencing a first cardiac event was greatest during ages 10 to 25 years, with the rate of first events less frequent thereafter. Each family had a few individuals who died suddenly at a young age of suspected LQTS-related death before the family was identified as having LQTS. These subjects did not have genotype data or an ECG. When these subjects were included in the life-table analyses for first cardiac events, the findings were similar to that observed among identified carriers shown in Figure 3. The clinical characteristics of carriers with and without cardiac events by age 40 are presented in Table 6. Carriers with events had more prolonged QT and QTc intervals than carriers without events ($P < 0.05$).

DISCUSSION

The main finding of this study is that the expression of the clinical phenotype of LQTS was as variable among family members with the same HERG gene mutation as it was among members of families with different mutations of the HERG gene. Evidence for variable expression includes diverse intra- and interfamilial morphological T-wave patterns and QTc intervals that substantially overlap normal values in each mutation. Although mean QTc values did not differ significantly among carriers with the five different mutations, the QTc distribution by mutation was quite variable. The variable expression in LQTS phenotype demonstrated in this study does not convey the complete spectrum of clinical phenotype since the study population included only five HERG-mutations (one N-terminus and four C-terminus mutations) out of the many that have been identified to date.¹⁴ No subjects with pore mutations were included in this study.

It is interesting that all four C-terminus mutations involved trafficking defects during in vitro homozygous expression carried out at physiologic

Table 3. QTc Variability Over Time by Mutation

Mutation	N	Months*	Delta QTc (msec)†
M124R	4	83 ± 59	-30 ± 70
R752W	3	29 ± 3	-40 ± 20
F805C	6	50 ± 32	10 ± 20
V822M	11	76 ± 56	-10 ± 40
W1001stop	13	75 ± 78	-40 ± 60

Plus-minus values are mean ± SD. *Time interval between first and last recorded ECG. † Difference in QTc interval between the first and last recorder electrocardiogram.

Table 4. Performance of QTc Categorization* Among 280 Genotyped Family Members by Mutation

Parameter	HERG Mutation					
	M124R	R752W	F805C	V822M	W1001stop	All
Sensitivity†	91 (10/11)	86 (12/14)	100 (7/7)	82 (14/17)	88 (15/17)	88 (58/66)
Specificity†	88 (15/17)	97 (60/62)	100 (6/6)	94 (15/16)	98 (49/50)	96 (145/151)
Positive predictive accuracy†	83 (10/12)	86 (12/14)	100 (7/7)	93 (14/15)	94 (15/16)	84 (58/64)
Negative predictive accuracy†	94 (15/16)	97 (60/62)	100 (6/6)	83 (15/18)	96 (49/51)	95 (145/153)
Equivocal ECG‡						
Frequency	18 (6/34)	26 (27/103)	7 (1/14)	27 (12/45)	20 (17/84)	23 (63/280)
Equivocal with gene mutation	50 (3/6)	48 (13/27)	0 (0/1)	33 (4/12)	6 (1/17)	33 (21/63)

All values are % (n).

* QTc categorization criteria¹⁵: affected = QTc > 460 msec for children 1–15 years of age, QTc > 450 msec for males > 15 years of age, and QTc > 460 for females > 15 years of age; unaffected = QTc < 440 msec for children 1–15 years of age, QTc < 430 msec for males > 15 years of age, and QTc < 450 msec for females > 15 years of age; equivocal = those not categorized as affected or unaffected by these QTc criteria.

† Calculations of sensitivity, specificity, positive and negative predictive accuracy excluded family members with equivocal (borderline) ECGs. Sensitivity = number of mutation-carriers with QTc affected/all mutation-carriers; Specificity = number of noncarriers with QTc unaffected/all noncarriers; Positive predictive accuracy = number of carriers with QTc affected/all QTc affected; Negative predictive accuracy = number of noncarriers with QTc unaffected/all QTc unaffected.

‡ Equivocal ECG = frequency-number equivocal ECGs/all ECGs; Equivocal with gene mutation-number of mutation-carriers with equivocal QTc/all QTc equivocal.

temperatures (Table 1). All the affected subjects in this study were heterozygous for the HERG mutations. In carriers with different HERG mutations, the magnitude of the reduced I_{Kr} current may be quite variable depending on the makeup and function of the co-assembled heterotetrameric channel protein involving normal and mutant subunits, as well as the actual number of functional channels located in the plasma membrane.

The QTc categorization scheme that we used included a subset with an equivocal (borderline) QTc interval. The overall performance of the QTc classification scheme for identifying subjects as affected or not affected was consistent by mutation,

mainly because the equivocal phenotype was excluded in the categorization. The finding of a relatively high rate (12%) of syncope among noncarriers, mostly in one family,¹⁹ reflects the relative softness of syncope as an endpoint and/or the presence of undetected LQTS mutations in these subjects.²⁰

Each gene mutation was associated with a spectrum of QTc values, and similar findings have been reported in one large pedigree with a single mutation involving the LQT1 gene.²¹ QTc is a continuous phenotypic trait, and the QTc cut-off value for defining an abnormally prolonged QTc interval is quite variable, ranging from 440 to 470 ms in var-

Table 5. Variability of LQT2 Phenotype in Carriers by Mutation

QTc, msec	HERG Mutation					
	M124R (n = 14)	R752W (n = 27)	F805C (n = 7)	V822M (n = 21)	W1001stop (n = 18)	All (n = 87)
Range, msec	420–710	410–540	460–560	420–590	420–590	410–710
< 440, n (%)	1 (7)	3 (11)	0 (0)	6 (29)	2 (11)	12 (14)
440–550, n (%)	10 (71)	24 (89)	6 (86)	13 (62)	12 (67)	65 (75)
> 550, n (%)	3 (21)	0 (0)	1 (14)	2 (10)	4 (22)	10 (11)
< 440 with events, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
440–550 with events, n (%)	5 (50)	6 (25)	3 (50)	2 (15)	5 (42)	21 (32)
550 with events, n (%)	2 (67)	0 (0)	0 (0)	1 (50)	3 (75)	6 (60)

Figure 2. QTc distribution and cardiac events in carriers (A) and noncarriers (B) from five families with different HERG mutations. Among the HERG carriers (A), the cardiac events (circles) include syncope, aborted cardiac arrest, or LQTS-related death. Among non-carriers of the HERG mutations (B), the only cardiac events (circles) that occurred were syncopal events.

ious reports, with influence of age, sex, and other factors on QTc. In a recent study of nine small families with mutations involving LQT1 or LQT2 genes, the average penetrance in these families was low, only 25%, using standard QTc and clinical-symptom criteria.²² We have used the phrase variable expression and have avoided the terms incomplete or low penetrance since these terms in LQTS assume an accurate dichotomy of QTc into normal and prolonged values.

CONCLUSION

The variable QTc intervals and T-wave patterns in carriers with the same gene mutation and the relatively stable QTc values over time in individual carriers suggest the influence of modifier genes on the phenotypic expression of LQTS in each of the five families with different HERG mutations. It is

Table 6. Clinical Characteristics by Outcome* in Carriers with HERG Mutations

Carrier Characteristic	Without LQTS Events (n = 68)	With LQTS Events (n = 28)
Female (%)	44	64
Age at 1 st ECG, years	31 ± 23	32 ± 16
Beta blockers, at baseline (%)	3	14*
Beta blockers, at < 40 years (%)	26	54*
Pacemaker, any time (%)	2	18*
LCTSG, any time (%)	0	4
ICD, any time (%)	0	0
ECG parameters at baseline (mean ± SD)		
RR, msec	831 ± 222	903 ± 175
QTpeak, msec	324 ± 54	374 ± 76
QT, msec	427 ± 65	476 ± 92†
QTc, msec	475 ± 44	501 ± 64†

ICD = implantable cardioverter defibrillator; LCTSG = left cervico-thoracic stellate ganglionectomy; *Outcome by age 40 years; †P < 0.05.

Figure 3. Cumulative probability of a first cardiac event using birth as the time origin among HERG carriers. The P value is for difference in outcomes among the five families.

difficult to evaluate the role of environmental factors in modifying the expression of LQTS in these families, but the stability of the QTc interval over several years suggests that the environmental influence is having only a small effect.

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