European Heart Journal Advance Access published December 28, 2015

European Heart Journal
doi:10.1093/eurheartj/ehv695

CLINICAL RESEARCH
Arrhythmia/electrophysiology

The genetics underlying acquired long QT syndrome: impact for genetic screening

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Received 25 February 2015; revised 18 November 2015; accepted 1 December 2015

Aims Acquired long QT syndrome (aLQTS) exhibits QT prolongation and Torsades de Pointes ventricular tachycardia triggered by drugs, hypokalaemia, or bradycardia. Sometimes, QTc remains prolonged despite elimination of triggers, suggesting the presence of an underlying genetic substrate. In aLQTS subjects, we assessed the prevalence of mutations in major LQTS genes and their probability of being carriers of a disease-causing genetic variant based on clinical factors.

Methods and results We screened for the five major LQTS genes among 188 aLQTS probands (55 ± 20 years, 140 females) from Japan, France, and Italy. Based on control QTc (without triggers), subjects were designated 'true aLQTS' (QTc within normal limits) or 'unmasked cLQTS' (all others) and compared for QTc and genetics with 2379 members of 1010 genotyped congenital long QT syndrome (cLQTS) families. Cardiac symptoms were present in 86% of aLQTS subjects. Control QTc of aLQTS was 453 ± 39 ms, shorter than in cLQTS (478 ± 46 ms, $P < 0.001$) and longer than in non-carriers (406 ± 26 ms, $P < 0.001$). In 53 (28%) aLQTS subjects, 47 disease-causing mutations were identified. Compared with cLQTS, in 'true aLQTS', *KCNQ1* mutations were much less frequent than *KCNH2* (20% [95% CI 7–41%] vs. 64% [95% CI 43–82%], $P < 0.01$). A clinical score based on control QTc, age, and symptoms allowed identification of patients more likely to carry LQTS mutations.

Conclusion A third of aLQTS patients carry cLQTS mutations, those on *KCNH2* being more common. The probability of being a carrier of cLQTS disease-causing mutations can be predicted by simple clinical parameters, thus allowing possibly cost-effective genetic testing leading to cascade screening for identification of additional at-risk family members.

Keywords Congenital long QT syndrome • Acquired long QT syndrome • Drug-induced long QT syndrome • Genetics

Introduction

Acquired long QT syndrome (aLQTS) is characterized by QT prolongation and sometimes Torsades de Pointes (TdP) ventricular tachycardia, and is provoked by the presence of QT-prolonging drugs,[1,2] hypokalaemia,[3] or bradycardia.[4] Culprit agents in aLQTS include many in common use, such as antihistamines, antibiotics, antidepressants, prokinetics, and many others.[1] The importance of

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aLQTS depends on its two main consequences: the unacceptable risk of sudden death when being medically treated for a non-life-threatening condition, and the implications for new drug development as many potentially useful agents are withdrawn from the market following a small number of cases of TdP.[1] The problem of drug-induced aLQTS has not been solved despite the efforts made by the Food and Drug Administration,[5–7] including strict non-clinical (S7B) and clinical (ICH E14) tests.

While, in most aLQTS cases, the QT interval returns to normal after the discontinuation of drug or other causative trigger, in some cases it remains prolonged. Partly on this basis, since 1982[8] it had been suggested that aLQTS may share some genetic background with congenital long QT syndrome (cLQTS).[9–11] In LQTS, 16 disease-causing genes have been identified so far, mostly encoding cardiac ion channel subunits or associated proteins.[12] Individual genetic background could be important, even in the presence of modest QT prolongation, because in association with appropriate triggers, it may favour the life-threatening manifestations of aLQTS. In this international collaborative study, we analysed a cohort of 188 aLQTS cases, assessed the prevalence of mutations in the major LQTS genes when compared with that in 1010 cLQTS probands, and evaluated the association of clinical factors, including QTc, with the probability of aLQTS subjects being carriers of a disease-causing genetic variant.

Methods

Study population

A total of 188 subjects diagnosed with aLQTS were identified at the participating centres in France ($n = 20$), Italy ($n = 21$), and Japan ($n = 147$). Patients were considered symptomatic if they exhibited TdP ventricular tachycardia, pre-syncope, syncope, cardiac arrest, or ventricular fibrillation, or as asymptomatic if they had a prolonged QTc (QT interval corrected for heart rate according to Bazett's formula[13] $\geq$480 ms) in the presence of proarrhythmic determinants.

QTc measurement

The QTc of aLQTS was measured in the presence and absence ('control QTc') of triggering factors. Control QTc was recorded either before or after an adequate period of time had elapsed from the critical event to allow washout and/or correction of the triggering condition. Depending on the value of control QTc, aLQTS subjects were retrospectively divided into two groups: 'true aLQTS' (QTc $<$460 ms in females and $<$450 ms in males) or 'unmasked cLQTS' (all non-true aLQTS). Control QTc in aLQTS was also compared with that of 2379 genotyped members (1938 carriers and 441 non-carriers) of 1010 cLQTS (Romano-Ward) families recruited in Japan ($n = 435$), France ($n = 296$), and Italy ($n = 279$).

Genetic analysis

The protocol for genetic analysis was approved by and performed under the guidelines of each institutional Ethics Committee. Written informed consent to participate in the study including consent for the collection and the use of DNA samples for genetic analysis was obtained in each centre. Genomic DNA was isolated from peripheral white blood cells using conventional methods. Genetic screening was performed for *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* genes, corresponding to LQT1, LQT2, LQT3, LQT5, and LQT6, in each centre. All index cases were tested; family members were analysed only if the proband had a mutation. We considered as disease-causing those mutations for which a link with LQTS has already been established in previous publications. In the absence of references, novel variants were included when deemed to be pathogenic or likely pathogenic applying the recently published ACMG guidelines,[14] which are based on criteria using typical types of variant evidence, including frequencies in population data and computational (*in silico*) predictive programmes. Two KCNH2 novel variants of uncertain significance according to ACMG criteria were included because they had never been described in any control population and both were present in the same subject, making very unlikely that such combined heterozygosity had not contributed to the clinical manifestations. Common polymorphisms and rare variants in accordance with previous reports were excluded.[15,16] Nevertheless, given its role as a QT modulator and a risk factor in cLQTS and aLQTS,[17,18] we also examined the frequency of the common variant *KCNQ1*-D85N.

Scoring system

We developed and assessed the performance of a composite score in predicting the probability of being a mutation carrier. Univariate analyses were first used to derive those demographic/clinical variables shown to be significantly associated with the presence of a disease-causing mutation. Three significant ($P < 0.05$) variables were identified, i.e. age, QTc, and the presence of symptoms, which were then included in a multivariable logistic regression model with the genetic status [mutation carriers (MCs)/non-mutation carriers (NMCs)] as the dependent variable. These three predictors were treated as dichotomized variables: for age at the critical event, the predefined cut-off was 40 years, the usual time horizon for the exposure to the risk of a first cardiac event in cLQTS; for the QT interval, the cut-off was 440 ms, the traditional limit for ECG-based diagnosis of cLQTS; the third variable was the presence/absence of symptoms at the time of aLQTS diagnosis. The positive presence of each variable was specifically categorized (i.e. age $<$40 years; QTc $>$440 ms; history of cardiac events) conferred 1 point. Thus, the score made up by adding the points for each factor present ranged from 0 to 3 points. We also considered the relative magnitude of the specific contribution of the three predictors and tested in a sensitivity analyses a 'weighted' version of the score. Specifically, points were derived from the final logistic regression model beta-coefficients and were assigned to each factor by dividing each beta coefficient by the smallest beta coefficient in the model and rounding to the nearest integer. Thus, this weighted scoring system assigned 1 point for age $<$40 years, 2 points for a QTc $>$440, and 3 points for a history of symptoms. As a result, each patient received a score ranging from 0 to 6 points. The goal was to identify a simple composite indicator of the probability of the presence of an LQTS mutation, based on features easily available to clinicians.

Statistical analysis

Normal continuous variables, expressed as mean $\pm$ standard deviation (SD), were compared by an unpaired *t*-test or by one-way ANOVA. Categorical variables, expressed as numbers and percentages, were analysed by χ^2 or Fisher's exact test, as appropriate. Binomial exact 95% confidence intervals (CIs) were computed to assess the reliability of the estimated proportions of mutation carriers. Multivariable logistic regression analysis was performed to evaluate the independent contribution of demographic and clinical variables to the probability of being mutation carriers among aLQTS individuals. All variables were tested for collinearity to exclude over-correlation with one another. Odds ratios with 95% CI are reported. To assess the discriminating power of the scoring system, i.e. its performance in distinguishing between aLQTS subjects likely to be or not mutation carriers, we used the receiving operator characteristic curve. A value of $P < 0.05$ was considered

statistically significant. SPSS Statistics version 21 (IBM Co, Armonk, NY, USA) was used for computation.

Results

Clinical background of acquired long QT syndrome

The larger aLQTS Japanese subset ($n = 147$) was not significantly different compared with the Caucasian one ($n = 39$) in all measured baseline clinical features, with the only exception of a somewhat older age. Among all the 188 aLQTS patients, females ($n = 140$, 74.5%) were prevalent and the mean age at the critical event was 55 ± 20 years. Most subjects ($n = 162$, 86%) were symptomatic; the remaining 26 (14%) came to medical attention because of a marked QT prolongation associated with secondary factors. These included: drugs ($n = 81$, Supplementary material online, *Table S1*), hypokalaemia (serum level <3.5 mEq/L, $n = 42$), bradycardia (mostly sick sinus syndrome or atrioventricular block, $n = 17$), a combination of at least two factors ($n = 43$), and 'other' factors ($n = 5$, three Takotsubo cardiomyopathy, one hypothermia, and one subarachnoid haemorrhage). When a distinction was made between drug-induced LQTS and all other causes of aLQTS, the two groups were comparable with respect to almost all baseline characteristics, the only exception being a significantly larger proportion of symptomatic and mutation carriers among those individuals with hypokalaemia, bradycardia, and 'other' factors as aLQTS determinants (*Table 1*). When aLQTS was secondary to hypokalaemia, the mean serum potassium concentration was 2.8 ± 0.5 mEq/L.

Latent QT prolongation in acquired long QT syndrome

The mean control QTc of the 188 aLQTS patients was 453 ± 39 ms, significantly shorter than that of the 1938 cLQTS MCs (478 ± 46 ms, $P < 0.001$) but longer than that of the 441 NMCs (406 ± 26 ms, $P < 0.001$). This suggests that already in the absence of any triggering factor, there is latent QTc prolongation (*Figure 1*). Based on their control QTc, 112 (60%) patients were true aLQTS and 76 (40%) were unmasked cLQTS. *Figure 2* shows representative ECGs of one patient of each subgroup. In association with the induced critical event, the QTc of aLQTS patients was dramatically prolonged (591 ± 82 ms).

Genetic background in acquired long QT syndrome

Genetic analysis identified 51 carriers of a single LQTS disease-causing mutation and 2 compound heterozygous carriers of genetic variants in different genes (*KCNQ1*/*KCNH2*) or in the same gene (*KCNH2*/*KCNH2*). Overall, 53 of the 188 aLQTS cases (28%, 95% CI 22–35) were found to be MCs following accidental exposure to an arrhythmia-triggering factor. A detailed list of all the 47 distinct mutations identified is presented in Supplementary material online, *Table S2*. Of these (all previously reported with the exception of 9 novel mutations), 13 were found in *KCNQ1*, 29 in *KCNH2*, 3 in *SCN5A*, 1 in *KCNE1*, and 1 in *KCNE2*. Mutations were found regardless of ethnicity, sex, or triggers, but were more frequently found in aLQTS patients diagnosed before age 40 than afterwards (41 vs. 24%, $P = 0.03$) and in symptomatic than in asymptomatic aLQTS (32 vs. 4%, $P = 0.002$; *Table 2*). Control QTc of the 53 aLQTS MCs was significantly longer than that observed in the 135 aLQTS NMCs (469 ± 36 vs. 447 ± 38 ms, $P < 0.001$), but comparable to the control QTc measured in the cLQTS MCs (478 ± 46 ms). Conversely, the QTc of aLQTS NMCs (447 ± 38) was more prolonged than that of cLQTS NMCs (406 ± 26, $P < 0.001$).

The analysis of aLQTS family members identified 56 mutation carriers (37 ± 24 years), with a mean QTc of 459 ± 36 ms. Of these 56 MCs, 54 (96%) were asymptomatic while 2 females with the R555C mutation in *KCNQ1* or the M124T mutation in *KCNH2* had

Table 1 Comparison between drug-induced LQTS and aLQTS from other causes

	Drug-induced aLQTS ($n = 117$)[a]	Non-drug-induced aLQTS ($n = 71$)	*P*-value
Females	83 (71)	57 (80)	0.15
Age (years)	55 ± 22	54 ± 19	0.64
QTc off trigger (ms)	451 ± 39	455 ± 39	0.49
True aLQTS	69 (59)	43 (61)	0.83
Unmasked LQTS	48 (41)	28 (39)	
QTc on trigger	585 ± 85	601 ± 76	0.23
Japanese : Caucasian : Black	91 (78) : 24 (20) : 2 (2)	56 (79) : 15 (21) : 0 (0)	0.54
Symptomatic	94 (80)	68 (96)	0.003
Mutation carriers	27 (23)	26 (37)	0.045
KCNQ1	9 (33)	6 (23)	0.167
KCNH2	13 (48)	17 (65)	
SCN5A	3 (11)	0	
KCNE1/*KCNE2*	2 (7)	1 (4)	
Double mutations	0	2 (8)	

Data are frequencies (%) or mean ± SD.
LQTS, long QT syndrome; aLQTS, acquired long QT syndrome.
[a]QT drugs only ($n = 81$) and QT drugs in association with hypokalaemia ($n = 27$) or bradycardia ($n = 9$).

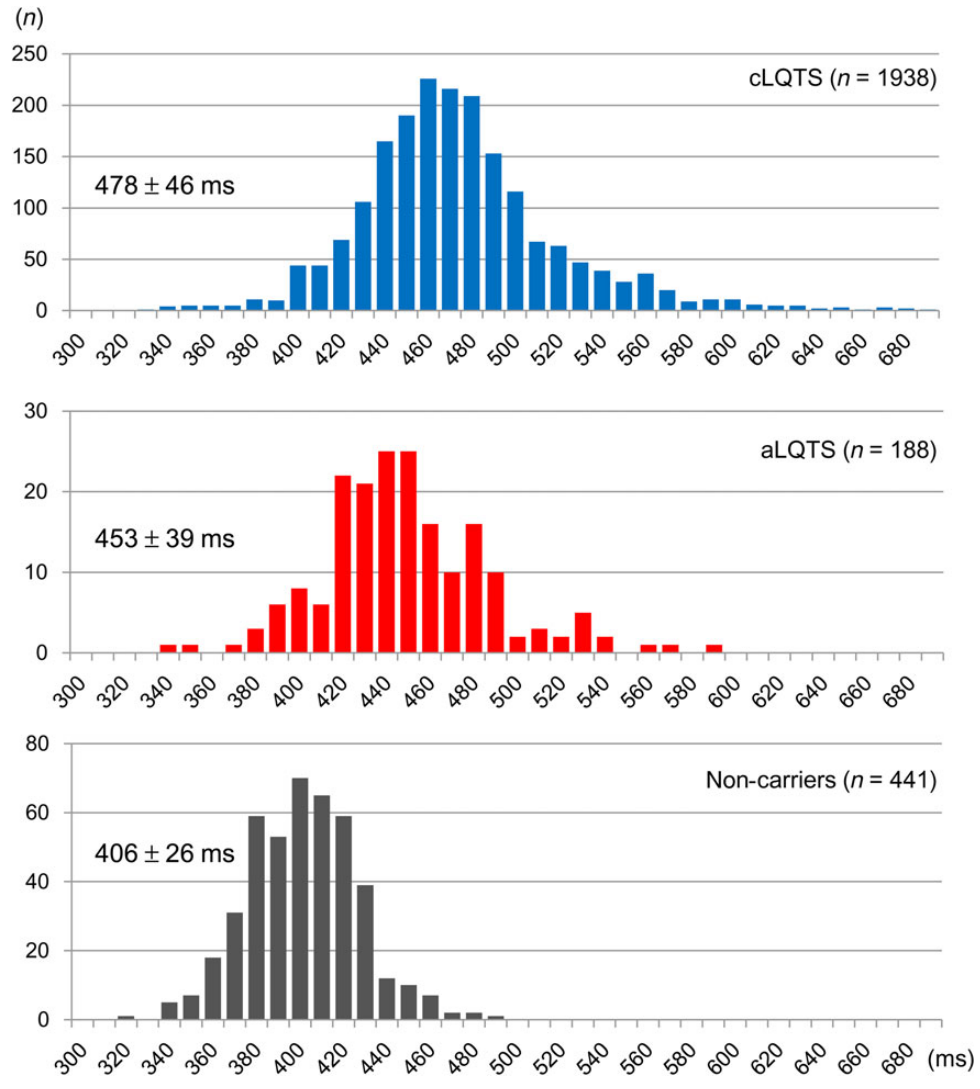

Figure 1 Distribution of QTc interval in genotyped congenital long QT syndrome, acquired long QT syndrome, and non-carriers.

cardiac events with prolonged QTc of 628 and 678 ms, respectively, while taking terfenadine or probucol; they had not been diagnosed as 'cLQTS' prior to these cardiac events.[9,19]

Mutations in acquired long QT syndrome and congenital long QT syndrome

To assess the relative frequencies of the LQTS genetic subtypes, we focused on single mutation carriers only, after exclusion of the 55 (2 aLQTS and 53 cLQTS) compound heterozygous MCs. Distribution of the LQTS genetic subtypes differed significantly between aLQTS and cLQTS (*Figure 3A*). Among the 957 cLQTS MCs, *KCNQ1* and *KCNH2* mutations were found in 477 (50% [95% CI 47–53%]) and 397 (41% [95% CI 38–45%]) patients, respectively. In contrast, among the 51 aLQTS individuals, carriers of *KCNQ1* mutations ($n = 15$, 29% [95% CI 17–44%]) were significantly less frequent than *KCNH2* MCs ($n = 30$, 59% [95% CI 44–72%]) compared with cLQTS ($P < 0.01$). The larger proportion of *KCNH2* MCs observed in the aLQTS cohort compared with cLQTS was independent of specific ethnic background, as it was comparable in the Japanese and Caucasian subsets [22/35 (63%) and 8/16 (50%), respectively]. It was also present both in the drug-induced LQTS and in the 'other' cause-aLQTS subset (*Table 1*). This overrepresentation of the LQT2 subtype with respect to cLQTS was more evident in true aLQTS than in the unmasked cLQTS subtype in which the percentages of *KCNQ1* (38%) and *KCNH2* (54%) MCs were intermediate between true aLQTS and cLQTS MCs (*Figure 3B*).

Interestingly, despite the small number of MCs in each genetic subgroup, *KCNE1*/*KCNE2* mutations seemed more frequent in aLQTS than in cLQTS (5.9% [95% CI 1.2–16%] vs. 1.7% [95% CI 1–2.7%], $P = 0.07$).

The common variant *KCNQ1*-D85N was found in 10/188 (5%) heterozygous subjects, corresponding to a minor allele frequency of 2.7%. Five of these were also carriers of *KCNH2* mutations. No significant difference was observed between aLQTS subjects with or without the D85N variant with respect to all demographic and

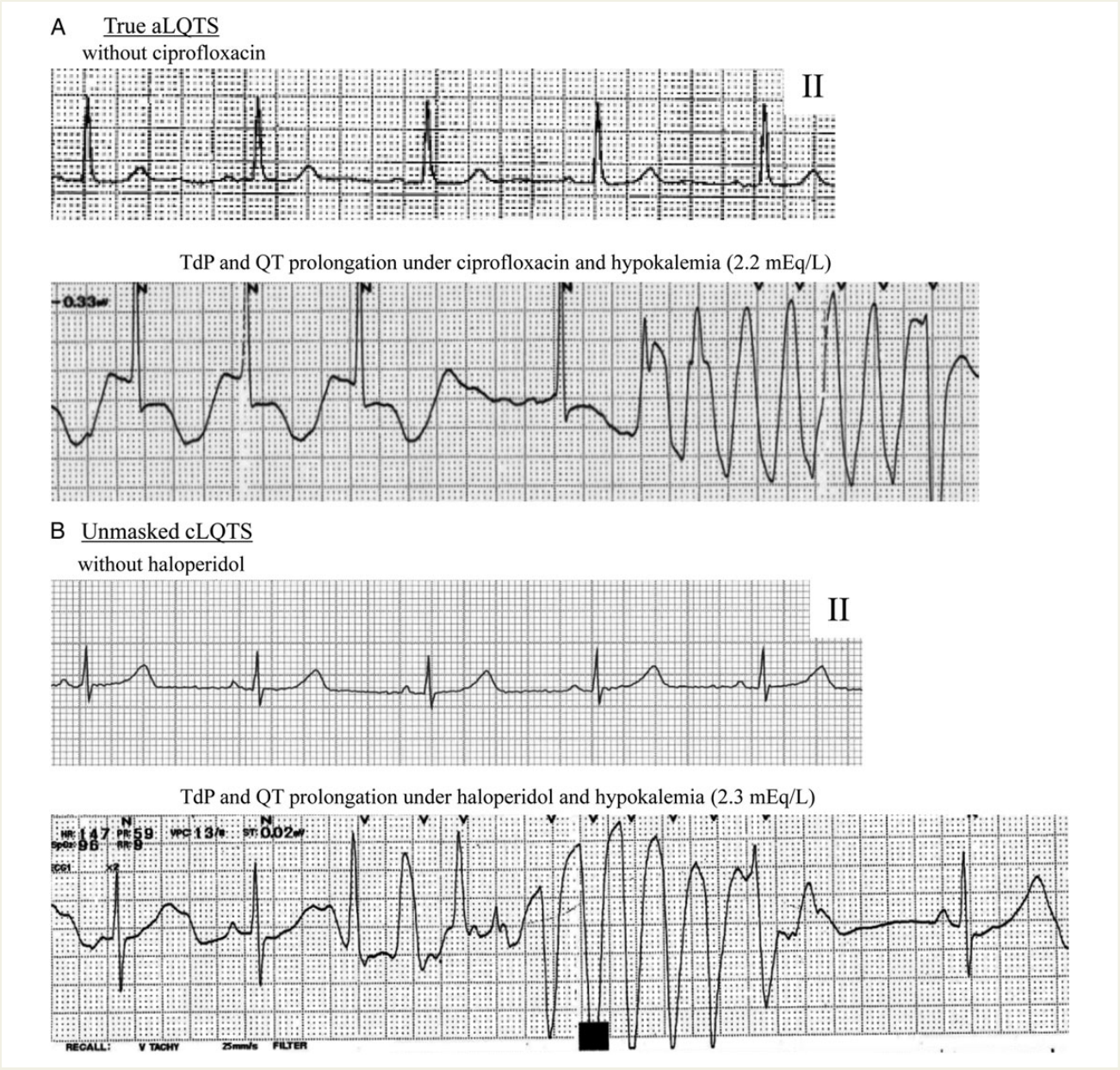

Figure 2 Electrocardiograms of true acquired long QT syndrome and unmasked congenital long QT syndrome. (*A*) A representative case with true long QT syndrome. This 73-year-old female was admitted for the treatment of bacterial pneumonia. Her basal QTc was normal (396 ms, upper panel). Torsades de Pointes and severe QT prolongation appeared 3 days after receiving ciprofloxacin (lower panel) and led to ventricular fibrillation. After cardioversion, the ECG showed marked QT prolongation (613 ms) and atrial fibrillation with hypokalaemia (2.2 mEq/L). (*B*) A representative case with unmasked congenital long QT syndrome. This 52-year-old alcoholic male was treated with haloperidol i.v. (6 mg) for withdrawal symptoms. Though his control QTc interval was prolonged but not remarkably (466 ms), a monitoring ECG showed Torsades de Pointes and marked QTc prolongation (624 ms) after haloperidol accompanied by hypokalaemia (2.3 mEq/L). Genetic analysis revealed a *KCNH2* mutation (R948S) located in the C-terminus.

clinical variables measured, although D85N carriers trended towards a longer control QTc (475 ± 38 vs. 452 ± 39 ms, $P = 0.067$) compared with non-carriers.

A score to predict genetic status

Using a multivariable logistic regression model, age at exposure to proarrhythmic triggers, control QTc measured in the absence of these factors, and a history of symptoms at the time of the critical event were significant and independent predictors for carriers of disease-causing mutations (*Table 3*). Their combination in a summative score ranging from 0 to 3 points allowed identification of individuals in whom genetic screening is more or less likely to reveal an LQTS mutation. The discriminatory power of the score was relatively good (AUC, 0.72, 95% CI 0.64–0.80, $P < 0.001$). As shown

Table 2 Prevalence of mutation carriers according to baseline features of the 188 aLQTS subjects

Demographic/clinical variable	Mutation carriers ($n = 55$)	*P*-value
Ethnicity		
Blacks	0/2	0.09
Caucasians	16/39 (41)	
Japanese	37/147 (25)	
Age of onset		
<40 years	20/49 (41)	0.03
≥40 years	33/139 (24)	
Sex		
Males	11/48 (23)	0.46
Females	42/140 (30)	
Secondary factors		
Drug	19/81 (23)	0.36
Hypokalaemia	17/42 (40)	
Bradycardia	5/17 (29)	
Combination	11/43 (26)	
Other	1/5 (20)	
Control QTc		
True aLQTS	26/112 (23)	0.07
Unmasked LQTS	27/76 (36)	
Cardiac symptoms		
Symptomatic	52/162 (32)	0.002
Asymptomatic	1/26 (4)	

Numbers are *n*/*N* (%).
LQTS, long QT syndrome; aLQTS, acquired long QT syndrome.

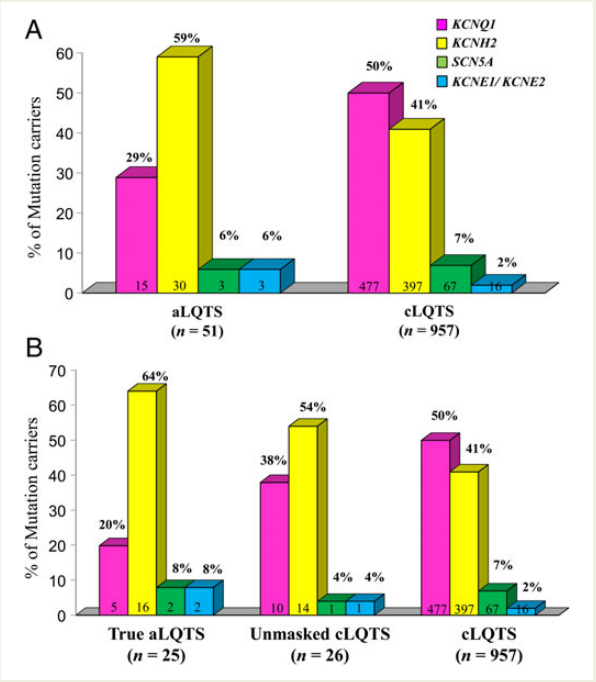

Figure 3 Distribution of genetic subtypes in acquired long QT syndrome and congenital long QT syndrome. All acquired long QT syndrome mutation carriers are shown in *A*; they are then subdivided in *B* as 'true acquired long QT syndrome' or 'unmasked congenital long QT syndrome', according to the study definitions.

Table 3 Predictors of the probability of being mutation carriers (MCs) in aLQTS

	OR (95% CI)	*P*-value
Age, <40 vs. ≥40 years	2.5 (1.2–5.3)	0.020
QTc, >440 vs. ≤440 ms	5.2 (2.2–12.2)	<0.001
Clinical status, symptomatic vs. asymptomatic	10.6 (1.3–83.5)	0.025

LQTS, long QT syndrome; aLQTS, acquired long QT syndrome.

in *Figure 4*, the proportion of aLQTS subjects with a positive genetic result increased linearly (*P* for trend < 0.001) from 0 to 63% (from 0 to 3 points), where all three factors conferring a higher probability (age ≤40 years, symptoms, and QTc >440 ms) were present. Of the aLQTS patients carrying a mutation, 89% (47 of 53) had a score of 2 or 3 points; from a different and broader perspective, of all the 188 aLQTS subjects none with a score of 0 and only 6 (3%) with a score of 1 had a mutation. The same significant pattern was present when examining separately Japanese and Caucasians (*Figure 5*).

When in a sort of sensitivity analysis we used a 'weighted' score by assigning 1 point to age <40 years, 2 points to QTc >440 ms, and 3 points to symptomatic status, the pattern was confirmed (see Supplementary material online, *Figure S1*).

Discussion

The present multicentre study focused on the genetic basis of aLQTS and provides four major findings: (i) the QTc measured in the absence of triggering factors of aLQTS cases is shorter than that of cLQTS patients, but is significantly longer than that of individuals without cLQTS; (ii) 28% of aLQTS subjects have mutations in cLQTS genes; (iii) at variance with cLQTS, the most prevalent mutations in aLQTS are on the *KCNH2* gene; and (iv) the QTc measured in the absence of triggers, together with simple clinical parameters, allows identification of aLQTS subjects more likely to be carriers of LQTS mutations and for whom, therefore, genetic screening is warranted.

Even though QTc returns to the normal range once QT-prolonging factors are removed,[20] previous reports observed that QTc sometimes remains prolonged even after withdrawal of culprit drugs[21] or the correction of serum electrolytes.[3] Our study cohort showed that the 'off' trigger QTc of aLQTS was indeed significantly longer than that of unaffected family members of cLQTS patients, in agreement with the concept that aLQTS could represent at least in part a latent genetic predisposition.[8] Our study now demonstrates in a large cohort of aLQTS cases that ~30% of these subjects have an

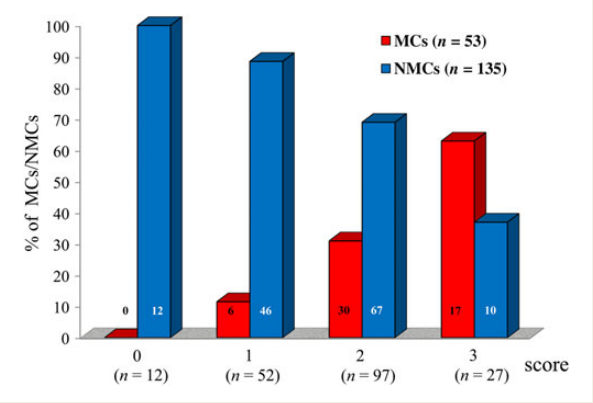

Figure 4 Proportions of mutation carriers/non-mutation carriers among the 188 patients according to increasing score values. Score 0 = age ≥40 years + asymptomatic + QTc ≤ 440 ms; score 3 = age <40 years + symptomatic + QTc >440 ms; Scores 1 and 2 represent the presence of one or of two factors. The number of mutation carriers increases with increasing score values (from 0 in the group with Score 0) and indicates that 89% of mutation carriers (47 of 53) are found within the Scores 2 and 3. Conversely, among the 52 patients with a score of 1, which represent 28% of the entire population, there were six mutation carriers; this means that while within the group with a score of 1, there is an 11% of mutation carriers, when looking at the entire population this percentage drops to 3%. This would be the percentage of mutation carriers missed if genetic screening would be limited to the groups with a score of 2 and 3.

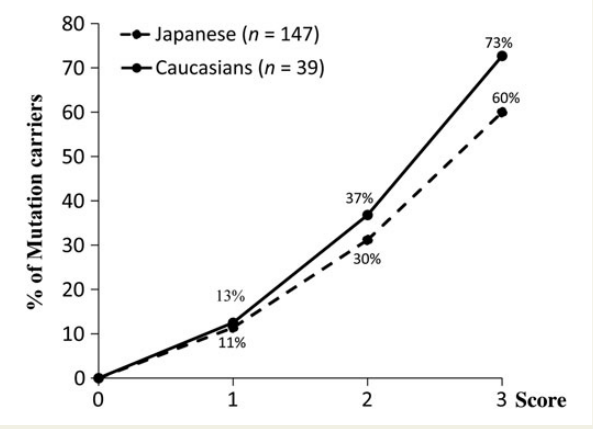

Figure 5 The percentages of mutation carriers, within each value of the probability score, are similar between Japanese and Caucasian acquired long QT syndrome subjects. The figures of 11 and 13% for a score of 1 correspond approximately at a 3% prevalence of mutation carriers on the total population of acquired long QT syndrome.

underlying mutation in one of the major LQTS-related genes. Even among the 'true aLQTS', i.e. those with normal QT interval outside the triggering episode, 23% had cLQTS-causing mutations and this percentage is much higher than the ~6% reported previously.[11,22]

We did consider the possibility that this greater prevalence, which was independent of ethnic background, might be due to the fact that our cohort included subjects whose aLQTS was caused not only by drugs, but also by hypokalaemia or bradycardia. However, this was not the case as the percentage of MCs among the drug-induced aLQTS was already high (23%) and the addition of non-drug-induced MCs increased this percentage.

The percentages of LQT1 and LQT2 patients in cLQTS are either similar or show a predominance of LQT1 mutations,[23,24] which was also the case in the present study. In contrast, in the 'true aLQTS', we observed a significantly lower frequency of LQT1- than LQT2-causing mutations. Even though it is unclear why *KCNH2* mutations are more frequently found in aLQTS, it may be relevant that most of these mutations are located in the non-pore regions, usually associated with rather benign phenotypes.[25] There was also a tendency for mutations in *KCNE1* and *KCNE2* to occur more frequently in aLQTS than in cLQTS. A possible explanation is that β-subunits are accessory proteins which modulate the function of the main α-subunits of the cardiac potassium channels and their altered channel dysfunctions are often benign and not sufficient *per se* to cause the full blown manifestations of cLQTS. Such considerations are clinically important because, in the absence of triggering factors, most of these mutations remain silent and the subjects appear normal.

No mutations were detected in two of three aLQTS patients. This does not necessarily rule out a genetic component, which may yet be identified. A number of rare or common polymorphisms could induce cumulative effects on QTc prolongation,[26] and even clinical phenotypes of long QT syndrome,[12] such as *KCNH2*-K897T,[27,28] *SCN5A*-S1103Y,[29] *KCNE1*-D85N,[17,18,28] and *NOS1AP*[30] variants. It is therefore tempting to speculate that the synergistic association of several functional polymorphisms could contribute to the genetic background predisposing to aLQTS. The D85N polymorphism, often associated with both cLQTS and aLQTS,[17,18,30] was not found in the present study to play any significant role despite a trend for longer QTc among the carriers.

The present findings go beyond clarification of the genetic basis for aLQTS because our analysis shows that a score incorporating simple parameters such as QTc, age, and presence/absence of symptoms may provide a useful guide for the clinician, assisting his/her decision-making and allowing cost-effective genetic testing.

Limitations

Genes responsible for the rarer variants of LQTS[12] were not analysed. Although their probability to cause cLQTS is very low (<1%), we cannot exclude the possibility that some genotype-negative aLQTS patients may have mutations in these genes or in others not yet identified. Our score lacks external or internal cross-validation and therefore it should be considered as a preliminary tool. However, the three variables used are well-known risk factors for arrhythmias in cLQTS, and their dichotomization was performed at traditional clinically based LQTS cut-offs, thus limiting the potential bias.

Conclusion

In clinical practice, greater attention should be paid to even mild prolongation of the QT interval because this could represent a signal of potential risk of manifestation of aLQTS if exposed to the

appropriate triggers. Some individuals initially labelled as aLQTS are diagnosed as cLQTS ('unmasked cLQTS') following the lack of QT normalization after removal of the trigger, and should be treated as such. Evidence of a genetic predisposition often present in aLQTS justifies and recommends a genetic study in all patients with a score of 2 or 3, where one finds almost 90% of the mutation carriers. While screening those with a score of 0 is unlikely to be useful, not to screen those with 1 point would miss only 3% of the subjects carrying a mutation within the entire aLQTS population and would be more debatable. Having identified the predisposing mutation in the proband, 'cascade screening' in their families will rapidly and inexpensively identify additional mutation carriers and prevent avoidable risks of life-threatening arrhythmias.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contributions

C.S., Y.M.: performed statistical analysis; P.J.S., H.I., L.C., W.S., M.H.: handled funding and supervision; H.I., L.C., T.A., I.D., V.F., K.H., T.N., S.O., T.M., J.W., K.H., E.M., F.D., M.P., M.Y., M.B., W.S., P.G., P.J.S., M.H.: acquired the data; P.J.S., L.C., C.S., P.G., M.H., H.I.: conceived and designed the research; P.J.S., H.I., C.S., M.H.: drafted the manuscript; P.J.S., L.C., C.S., M.H., H.I.: made critical revision of the manuscript for key intellectual content.

Acknowledgement

We thank Arisa Ikeda, Rika Kasahara, Tamayo Hirata, and Aya Umehara for their excellent technical assistance, and Pinuccia De Tomasi for her expert editorial support.

Funding

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (H.I., 25460406), the Japan Ministry of Health, Labour, and Welfare (W.S. and M.H., H24-033 and H26-040), the Japan Circulation Society (M.H., TR funds), and the Ministry of Education, Culture, Sports, Science and Technology (H.I., 25136705), National Natural Science Foundation of China (J.W., 81273501 and 81470378), the Italian Ministry of Education, University and Research (MIUR) Grants FIRB RBFR12I3KA (L.C.) and PRIN 2010BWY8E9 (P.J.S. and L.C.), and the Italian Ministry of Health Grant GR-2010-2305717 (L.C.) and Italian Ministry of Health Malattie Rare RF-IAI_2008-1216776 (P.J.S. and L.C.).

Conflict of interest: none declared.

References

1. Roden DM. Drug-induced prolongation of the QT interval. *N Engl J Med* 2004;**350**:1013–1022.
2. Wysowski DK, Bacsanyi J. Cisapride and fatal arrhythmia. *N Engl J Med* 1996;**335**:290–291.
3. Berthet M, Denjoy I, Donger C, Demay L, Hammoude H, Klug D, Schulze-Bahr E, Richard P, Funke H, Schwartz K, Coumel P, Hainque B, Guicheney P. C-terminal HERG mutations: the role of hypokalemia and a KCNQ1-associated mutation in cardiac event occurrence. *Circulation* 1999;**99**:1464–1470.
4. Kurita T, Ohe T, Marui N, Aihara N, Takaki H, Kamakura S, Matsuhisa M, Shimomura K. Bradycardia-induced abnormal QT prolongation in patients with complete atrioventricular block with Torsades de Pointes. *Am J Cardiol* 1992;**69**:628–633.
5. Food and Drug Administration HHS. International Conference on Harmonisation; guidance on S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals; availability. Notice. *Fed Regist* 2005;**70**:61133–61134.
6. Food and Drug Administration HHS. International Conference on Harmonisation; guidance on E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs; availability. Notice. *Fed Regist* 2005;**70**:61134–61135.
7. Darpo B, Garnett C, Benson CT, Keirns J, Leishman D, Malik M, Mehrotra N, Prasad K, Riley S, Rodriguez I, Sager P, Sarapa N, Wallis R. Cardiac Safety Research Consortium: can the thorough QT/QTc study be replaced by early QT assessment in routine clinical pharmacology studies? Scientific update and a research proposal for a path forward. *Am Heart J* 2014;**168**:262–272.
8. Moss AJ, Schwartz PJ. Delayed repolarization (QT or QTU prolongation) and malignant ventricular arrhythmias. *Mod Concepts Cardiovasc Dis* 1982;**51**:85–90.
9. Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M, Chivoret G, Schwartz K, Coumel P, Guicheney P. KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. *Circulation* 1997;**96**:2778–2781.
10. Makita N, Horie M, Nakamura T, Ai T, Sasaki K, Yokoi H, Sakurai M, Sakuma I, Otani H, Sawa H, Kitabatake A. Drug-induced long-QT syndrome associated with a subclinical SCN5A mutation. *Circulation* 2002;**106**:1269–1274.
11. Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, Hohnloser SH, Shimizu W, Schwartz PJ, Stanton M, Murray KT, Norris K, George AL, Roden DM. Allelic variants in long-QT disease genes in patients with drug-associated Torsades de Pointes. *Circulation* 2002;**105**:1943–1948.
12. Schwartz PJ, Ackerman MJ, George AL, Wilde AA. Impact of genetics on the clinical management of channelopathies. *J Am Coll Cardiol* 2013;**62**:169–180.
13. Bazett HC. An analysis of the time relations of electrocardiograms. *Heart* 1920;**7**:353–370.
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;**17**:405–424.
15. Kapa S, Tester DJ, Salisbury BA, Harris-Kerr C, Pungliya MS, Alders M, Wilde AA, Ackerman MJ. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation* 2009;**120**:1752–1760.
16. Giudicessi JR, Kapplinger JD, Tester DJ, Alders M, Salisbury BA, Wilde AA, Ackerman MJ. Phylogenetic and physicochemical analyses enhance the classification of rare nonsynonymous single nucleotide variants in type 1 and 2 long-QT syndrome. *Circ Cardiovasc Genet* 2012;**5**:519–528.
17. Nishio Y, Makiyama T, Itoh H, Sakaguchi T, Ohno S, Gong YZ, Yamamoto S, Ozawa T, Ding WG, Toyoda F, Kawamura M, Akao M, Matsuura H, Kimura T, Kita T, Horie M. D85N, a KCNE1 polymorphism, is a disease-causing gene variant in long QT syndrome. *J Am Coll Cardiol* 2009;**54**:812–819.
18. Kääb S, Crawford DC, Sinner MF, Behr ER, Kannankeril PJ, Wilde AA, Bezzina CR, Schulze-Bahr E, Guicheney P, Bishopric NH, Myerburg RJ, Schott JJ, Pfeufer A, Beckmann BM, Martens E, Zhang T, Stallmeyer B, Zumhagen S, Denjoy I, Bardai A, Van Gelder IC, Jamshidi Y, Dalageorgou C, Marshall V, Jeffery S, Shakir S, Camm AJ, Steinbeck G, Perz S, Lichtner P, Meitinger T, Peters A, Wichmann HE, Ingram C, Bradford Y, Carter S, Norris K, Ritchie MD, George AL, Roden DM. A large candidate gene survey identifies the KCNE1 D85N polymorphism as a possible modulator of drug-induced torsades de pointes. *Circ Cardiovasc Genet* 2012;**5**:91–99.
19. Hayashi K, Shimizu M, Ino H, Yamaguchi M, Terai H, Hoshi N, Higashida H, Terashima N, Uno Y, Kanaya H, Mabuchi H. Probucol aggravates long QT syndrome associated with a novel missense mutation M124T in the N-terminus of HERG. *Clin Sci (Lond)* 2004;**107**:175–182.
20. Roden DM, Viswanathan PC. Genetics of acquired long QT syndrome. *J Clin Invest* 2005;**115**:2025–2032.
21. Itoh H, Sakaguchi T, Ding WG, Watanabe E, Watanabe I, Nishio Y, Makiyama T, Ohno S, Akao M, Higashi Y, Zenda N, Kubota T, Mori C, Okajima K, Haruna T, Miyamoto A, Kawamura M, Ishida K, Nagaoka I, Oka Y, Nakazawa Y, Yao T, Jo H, Sugimoto Y, Ashihara T, Hayashi H, Ito M, Imoto K, Matsuura H, Horie M. Latent genetic backgrounds and molecular pathogenesis in drug-induced long-QT syndrome. *Circ Arrhythm Electrophysiol* 2009;**2**:511–523.
22. Behr ER, Ritchie MD, Tanaka T, Kääb S, Crawford DC, Nicoletti P, Floratos A, Sinner MF, Kannankeril PJ, Wilde AA, Bezzina CR, Schulze-Bahr E, Zumhagen S, Guicheney P, Bishopric NH, Marshall V, Shakir S, Dalageorgou C, Bevan S, Jamshidi Y, Bastiaenen R, Myerburg RJ, Schott JJ, Camm AJ, Steinbeck G, Norris K, Altman RB, Tatonetti NP, Jeffery S, Kubo M, Nakamura Y, Shen Y, George AL, Roden DM. Genome wide analysis of drug-induced Torsades de Pointes: lack of common variants with large effect sizes. *PLoS One* 2013;**8**:e78511.

23. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D. Risk stratification in the long-QT syndrome. *N Engl J Med* 2003;**348**:1866–1874.
24. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Heart Rhythm* 2005;**2**:507–517.
25. Shimizu W, Moss AJ, Wilde AA, Towbin JA, Ackerman MJ, January CT, Tester DJ, Zareba W, Robinson JL, Qi M, Vincent GM, Kaufman ES, Hofman N, Noda T, Kamakura S, Miyamoto Y, Shah S, Amin V, Goldenberg I, Andrews ML, McNitt S. Genotype-phenotype aspects of type 2 long QT syndrome. *J Am Coll Cardiol* 2009;**54**:2052–2062.
26. Newton-Cheh C, Eijgelsheim M, Rice KM, de Bakker PI, Yin X, Estrada K, Bis JC, Marciante K, Rivadeneira F, Noseworthy PA, Sotoodehnia N, Smith NL, Rotter JI, Kors JA, Witteman JC, Hofman A, Heckbert SR, O'Donnell CJ, Uitterlinden AG, Psaty BM, Lumley T, Larson MG, Stricker BH. Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nat Genet* 2009;**41**:399–406.
27. Crotti L, Lundquist AL, Insolia R, Pedrazzini M, Ferrandi C, De Ferrari GM, Vicentini A, Yang P, Roden DM, George AL, Schwartz PJ. KCNH2-K897T is a genetic modifier of latent congenital long-QT syndrome. *Circulation* 2005;**112**:1251–1258.
28. Gouas L, Nicaud V, Berthet M, Forhan A, Tiret L, Balkau B, Guicheney P, Group DE-SIRS. Association of KCNQ1, KCNE1, KCNH2 and SCN5A polymorphisms with QTc interval length in a healthy population. *Eur J Hum Genet* 2005;**13**:1213–1222.
29. Van Norstrand DW, Tester DJ, Ackerman MJ. Overrepresentation of the proarrhythmic, sudden death predisposing sodium channel polymorphism S1103Y in a population-based cohort of African-American sudden infant death syndrome. *Heart Rhythm* 2008;**5**:712–715.
30. Crotti L, Monti MC, Insolia R, Peljto A, Goosen A, Brink PA, Greenberg DA, Schwartz PJ, George AL. NOS1AP is a genetic modifier of the long-QT syndrome. *Circulation* 2009;**120**:1657–1663.