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Torsades de pointes following acute myocardial infarction: Evidence for a deadly link with a common genetic variant

Lia Crotti, MD, PhD^{*,†,‡}, Dan Hu, MD, PhD[§], Hector Barajas-Martinez, PhD[§], Gaetano M. De Ferrari, MD[†], Antonio Oliva, MD, PhD^{§,||}, Roberto Insolia, PhD^{*,†}, Guido D. Pollevick, PhD[§], Federica Dagradi, PhD^{*,†}, Alejandra Guerchicoff, PhD[§], Federica Greco, BSc^{*,†}, Peter J. Schwartz, MD, FACC, FAHA, FESC, FHR^{*,†,||,#,**}, Sami Viskin, MD^{††}, and Charles Antzelevitch, PhD, FHR[§], FACC, FAHA[§]

^{*}Section of Cardiology, Department of Molecular Medicine, University of Pavia, Pavia, Italy

[†]Department of Cardiology, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy

[‡]Institute of Human Genetics, Helmholtz Center Munich, Neuherberg, Germany

[§]Masonic Medical Research Laboratory, Utica, New York

^{||}Institute of Legal Medicine, Catholic University, School of Medicine, Rome, Italy

^{††}Cardiovascular Genetics Laboratory, Hatter Institute for Cardiovascular Research in Africa, Department of Medicine, University of Cape Town, South Africa

[#]Department of Medicine, University of Stellenbosch, Tygerberg, South Africa

^{**}Chair of Sudden Death, Department of Family and Community Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia

^{††}Tel-Aviv Sourasky Medical Center and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Abstract

BACKGROUND—Although QT prolongation following myocardial infarction (MI) is generally moderate, cases with marked QT prolongation leading to life-threatening torsades de pointes (TdP) have been described.

OBJECTIVE—To investigate the genetic substrate of this phenomenon.

METHODS—We studied 13 patients who developed TdP in the subacute phase of MI (2–11 days) and a group of 133 ethnically matched controls with uncomplicated MI. Long QT syndrome genes and the *KCNH2*-K897T polymorphism were screened by using denaturing high-performance liquid chromatography plus direct sequencing and a specific TaqMan assay, respectively.

RESULTS—Two of the 13 patients (15%) who presented with QT prolongation and TdP were found to carry long QT syndrome mutations (*KCNH2*-R744X and *SCN5A*-E446K). Nine of the remaining 11 patients (82%) carried the *KCNH2*-K897T polymorphism, which was present in 35% of the controls ($P = .0035$). Thus, patients with an acute MI carrying the *KCNH2*-K897T

polymorphism had an 8-fold greater risk of experiencing TdP compared with controls (95% confidence interval = 2–40).

CONCLUSIONS—Our data suggest that the common K897T polymorphism is associated with an increased risk of TdP developing in the subacute phase of MI. Our findings support the concept that the electrical remodeling associated with this healing phase of MI may unmask a genetic substrate predisposing to a time-limited development of life-threatening arrhythmias. They also provide the first line of evidence in support of the hypothesis that a common polymorphism, previously described as a modifier of the severity of LQTS, may increase the risk of life-threatening arrhythmias in a much more prevalent cardiac disease such as myocardial infarction.

Keywords

Long QT syndrome; Cardiac arrhythmia; Sudden cardiac death; Molecular genetics; Single nucleotide polymorphism

Introduction

Sudden cardiac death (SCD) is a leading cause of mortality in adults, and in Western cultures coronary heart disease accounts for at least 80% of these deaths.¹ There is growing evidence suggesting a genetic predisposition for SCD in this setting.^{2–6}

The period following myocardial infarction (MI) is often complicated by the development of life-threatening arrhythmias, sometimes associated with the appearance of prolonged QT intervals. We and others demonstrated that post-MI patients who continue to have a prolongation of the QT interval on their electrocardiograms (ECGs) are at a significantly higher risk of dying suddenly,⁷ a finding recently confirmed by Chugh and associates.⁸ QT prolongation as a marker of sudden death has gained interest in the genetic era because of the realization that a multiplicity of gene variants, rare and common, mostly encoding currents involved in the ionic control of ventricular repolarization, can determine changes—some modest, some larger—in the duration of the QT interval likely to influence prognosis.⁹ An association between excessively prolonged QT intervals and potentially lethal torsades de pointes (TdP) ventricular tachycardia developing in patients recovering from MI was established by Viskin and coworkers¹⁰ in 2001, and its incidence was estimated to be 1.8%. The mechanism responsible has remained elusive.

The present study was designed to assess a possible genetic basis for the association between TdP and MI via targeted genetic screening of patients who after an acute MI developed episodes of life-threatening TdP in the setting of deep-inverted T waves and very prolonged QT interval. We targeted the genes most prevalent in inducing congenital long QT syndrome (LQTS). Also, in light of our demonstration that the common polymorphism *KCNH2*-K897T has the potential to interact synergistically with otherwise benign LQTS mutations and precipitate cardiac arrest and sudden death,^{11,12} we considered the hypothesis that the influence of this polymorphism may extend beyond the realm of influencing the functional expression of other genetic mutations. In these previous studies, we demonstrated that the underlying electrophysiological mechanism is secondary to the effect of the K897T polymorphism to accentuate the loss of function of delayed rectifier potassium current (I_{Kr}) caused by other mutations in *KCNH2*.^{11,12} In the present study, we hypothesized that K897T has the potential to deleteriously interact with other more common clinical conditions. In addition, given the recent evidence that 3 *NOS1AP* polymorphisms (rs10494366, rs16847548, and rs4657139) could act as genetic modifiers in LQTS,^{9,13} we screened for these variants.

Methods

Study population

The population under study included a cohort of 13 Caucasian cases presenting with all the following: (1) an acute MI diagnosed clinically and confirmed by serial ECGs (showing new ST-segment deviation or new Q waves) and by serial blood tests (showing high creatine kinase and creatine kinase-muscle type (M) brain type (B) fraction or troponin-I levels), (2) spontaneous pause-dependent TdP documented during obvious QT prolongation, and (3) no other known causes of LQTS (including medications known to affect repolarization, bradyarrhythmias, or metabolic abnormalities).

A group of 133 ethnically matched patients with a first acute MI and no arrhythmic events served as controls.

ECG analysis

All ECGs collected at day 5 post-MI were coded and analyzed by an investigator (F.D.) blind to genotype. In the 13 patients who developed TdPs, ECGs recorded not only at day 5 but also just before the onset of TdP were analyzed by 2 investigators (S.V. and F.D.). Baseline QT and RR intervals were measured from resting 12-lead ECGs. The QT interval was corrected for heart rate (QTc) by using the Bazett formula.

Genotyping

Informed consent was obtained by using a protocol approved by the respective ethics review boards. All coding exons and intron borders of the 5 LQTS genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*) were genetically screened. All exon and intron borders were amplified from genomic DNA by polymerase chain reaction by using previously published primers or custom-designed primers (sequences available on request) and directly sequenced from both directions by using ABI PRISM 3100 Automatic DNA sequencer (Applied Biosystems, Foster City, CA). In some cases, amplicons were screened by using denaturing high-performance liquid chromatography instruments (WaveTM 3500HT; Transgenomic, San Jose, CA). Products exhibiting divergent chromatographic profiles from normal control samples were then purified (ExoSAP-IT; Amersham Bioscience, Piscataway, NJ) and bidirectionally sequenced by using Big-Dye Terminator v1.1 kit and ABI Prism 310 Genetic Analyzer (Applied Biosystems). The genetic variants identified were probed in a population of 300 ethnically matched controls and in all available online databases.

Cases and controls were screened for the *KCNH2*-K897T polymorphism and for 3 *NOS1AP* polymorphisms (rs10494366, rs16847548, and rs4657139) by using the 5' nucleotidase TaqMan assays (ABI Prism 7900HT; Applied Biosystems). Genotyping results were randomly confirmed via direct sequencing.

Functional expression

The *SCN5A* mutation identified was expressed in TSA201 cells and functionally characterized by using whole-cell patch-clamp techniques. The *SCN5A* mutation was prepared by using the Gene Tailor Site-Directed Mutagenesis System (Invitrogen, Carlsbad, CA) on full-length wild-type (WT) *SCN5A* cDNA cloned into pcDNA3.1 + (Invitrogen).

Functional characterization of the mutation was conducted by coexpression of *SCN5A* and *SCN1B* in TSA201 cells. CD8 cDNA (0.25 µg) was cotransfected as a reporter gene to visually identify transfected cells by using Dynabeads (M-450 CD8; Dynal Biotech, Oslo, Norway). Whole cell currents were recorded by using patch-clamp techniques. Na⁺ current was recorded by using bath solution perfusion containing 140 mM/L NaCl, 5 mM/L KCl,

1.8 mM/L CaCl₂, 1 mM/L MgCl₂, 2.8 mM/L Na Acetate, 10 mM/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 10 mM/L glucose (pH 7.3 with NaOH). Tetraethylammonium (TEA) (5 mM/L) was added to the buffer. Patch pipettes were filled with a solution containing 5 mM/L NaCl, 5 mM/L KCl, 130 mM/L CsF, 1.0 mM/L MgCl₂, 5 mM/L Ethylenebis tetraacetic acid (EGTA), and 10 mM/L HEPES (pH 7.2 with CsOH). Currents were filtered with a 4-pole Bessel filter at 5 kHz and digitized at 50 kHz. Series resistance was electronically compensated at 70%–85%. All data acquisition and analysis were performed by using pCLAMP V9.2 (Axon Instruments, Foster City, CA), Excel (Microsoft, Redmond, WA), and ORIGIN 7.0 (Microcal Software, Northampton, MA).

Statistical analysis

Comparisons of groups identified on the basis of the clinical characteristics and genotype were performed in univariate analysis. The Student *t* test was used for continuous variables. The Fisher test was used for categorical variables, and odds ratios for unadjusted data and their 95% confidence intervals were calculated. QTc data are presented as mean $\pm$ standard deviation. As 4 single nucleotide polymorphisms (SNPs) were tested, a 2-sided *P* value of $<.0125$ ($0.05/4$) was considered statistically significant. Prism 3.02 (Graph-Pad Software, La Jolla, CA) was used for statistical analysis.

The Hardy-Weinberg equilibrium was calculated with the “Chi-square Hardy-Weinberg equilibrium test calculator for biallelic markers” (Copyright TRG, SR, INMD, 2008).¹⁴

For the functional characterization of the mutation identified on SCN5A, data are expressed as mean $\pm$ standard error of the mean. Two-tailed Student *t* test and analysis of variance coupled with a Tukey’s test were used for statistical analysis (Systat Software, Inc, San Jose, CA). A *P* value of $<.05$ was considered statistically significant.

Results

Study population

Thirteen patients (8 men and 5 women), aged 66 ± 18 years (range 33–88 years), developed marked QT prolongation and TdP following acute MI. Figure 1A illustrates the typical progression of the development of the phenotype in a 47-year-old man with an anterior MI. T-wave inversion beginning on day 2 progresses gradually with the development of deeply inverted T waves and prolonged QT intervals, culminating in an episode of pause-dependent TdP on day 11. The long QT phenotype was totally reversed at 2-month follow-up. Figure 1B illustrates another example of pause-dependent TdP in a patient with inferior MI. Of note, none of the patients had hypokalemia or hypomagnesemia, and medications prescribed before the onset of TdP did not include QT-prolonging drugs. However, their QTc just before the onset of TdP was above 480 ms in all patients (on average 650 ± 130 ms). The culprit MI was a Q-wave infarction in 12 patients and was located to the anterior wall in 46% of the cases. Left ventricular ejection fraction was $45\% \pm 13\%$, and reperfusion therapy (percutaneous coronary intervention/thrombolysis) was performed in 83% of the cases.

The control group included 133 ethnically matched patients with an MI not complicated by ventricular arrhythmias; their age was 60 ± 11 years and their QTc was 434 ± 41 ms on day 5 after the acute MI. The culprit MI involved the anterior wall in 47%, left ventricular ejection fraction was $48\% \pm 7\%$, and reperfusion therapy (percutaneous coronary intervention/thrombolysis) was performed in 88%. Cases and controls did not differ in age ($P = .15$), site of MI ($P = 1$), reperfusion therapy ($P = .47$), or left ventricular ejection fraction ($P = .36$); however, the QTc of TdP cases measured on day 5 post-MI (538 ± 72 ms) was significantly greater than the QTc of controls (434 ± 41 ms) on day 5 ($P < .01$).

LQTS screening

An LQTS mutation was identified in 2 of the 13 TdP patients screened (15%). The *KCNH2*-R744X mutation, previously described as a LQTS disease-causing mutation,^{15,16} was identified in a 67-year-old patient who experienced multiple episodes of pause-dependent TdP following coronary bypass graft surgery after an inferior MI. His personal and family history was negative for syncope or SCD. Interestingly, the identification of this mutation led to cascade screening^{17,18} in family members, and the same mutation was detected in a daughter and 2 grandsons (Figure 2A). They were all asymptomatic, but their basal ECG showed LQT2 morphology and QT intervals of 455–482 ms with abnormal QT prolongation in response to brisk standing.¹⁹ The R744X mutation in the C-terminal region of the human ether-a-go-go-related gene (HERG) channel is predicted to cause truncation of the protein and deletion of the putative cyclic nucleotide-binding domain, spanning amino acid residues 742–842 (Figures 2B and 2C). The *KCNH2* nonsense mutation was not functionally expressed because there are ample data to suggest that this mutation produces a loss of function in I_{Kr} . The proband's daughter and her 2 sons were screened after the mutation was uncovered in the proband; all 3 were found to carry the R744X mutation. Genotype-phenotype correlation was excellent, with all carriers showing a high probability of LQTS based on the clinical phenotype; all received therapy for LQTS. Additional evidence for a loss of function of this mutation is available in the literature.^{16,20} In the study by Schwartz and coworkers reporting on this LQTS mutation, 14 family members were studied and a perfect genotype-phenotype correlation was observed.¹⁶

A *SCN5A*-E446K mutation (Figure 3A) was identified in an 88-year-old patient who developed repeated bursts of TdP on the third and fourth days following acute MI. The E446K mutation, predicted to be in the intracellular linker between DI and DII of the $Na_v1.5$ channel (Figure 3B), is highly conserved among mammalian species and was not observed in 300 ethnically matched healthy controls (Figure 3C). The patient carrying this mutation had a QTc of 480 ms on admission, but on the third and fourth days post-MI, when TdP occurred, the QTc prolonged to a range between 510 and 600 ms.

None of the cases carried the KCNE1-D85N rare variant, recently identified as an important modulator of drug-induced TdP.²¹

Functional characterization of *SCN5A*-E446K

The *SCN5A*-E446K mutation was functionally characterized. The electrophysiological study demonstrated a significant alteration in gating characteristics. Peak E446K sodium current was 26.51% smaller than WT following a step to -35 mV from a holding potential of -120 mV ($n = 33$ and 22 for WT and E446K, respectively; $P < .05$; Figures 3D–3E). For activation, $V_{1/2} = -50.81 \pm 1.80$ mV and $k = 6.88 \pm 1.62$ mV for WT and $V_{1/2} = -51.52 \pm 1.02$ mV and $k = 6.06 \pm 0.90$ mV for E446K. There were no significant differences in $V_{1/2}$ and k . The E446K mutant showed marked acceleration of current decay and a -6.16 -mV shift of steady-state inactivation ($n = 17$ and 20 for WT and E446K, respectively; $V_{1/2} = -92.53 \pm 1.02$ mV and $k = 5.24 \pm 0.90$ mV for WT and $V_{1/2} = -98.69 \pm 1.63$ mV and $k = 6.24 \pm 0.91$ mV for E446K; $P < .05$ for differences in $V_{1/2}$, but $P > .05$ in k ; Figures 3F and 3G). Using a standard double paired-pulse protocol, the rate of recovery from inactivation was shown to be slower for E446K channels than for WT. Double-exponential fits yielded the following time constants: $\tau_f = 6.72 \pm 1.27$ ms, $\tau_s = 50.35 \pm 4.25$ ms for WT ($n = 70$); $\tau_f = 10.76 \pm 1.33$ ms, $\tau_s = 48.29 \pm 3.89$ ms for E446K ($n = 21$); $P < .05$ for difference in τ_f , but τ_s and fractional amplitudes were not significantly different as compared with WT. E446K channels also exhibited an increased late sodium channel. Late inward sodium-channel current amplitude at 300-ms depolarization was $0.142\% \pm 0.018\%$ ($n = 22$) of peak inward

current for WT channels and $0.223\% \pm 0.028\%$ ($n = 9$) for E446K mutant channels ($P < .05$; Figures 3H and 3I).

KCNH2-K897T genotyping

The *KCNH2*-K897T polymorphism was investigated in all TdP cases ($n = 13$) and in 133 ethnically matched controls with uncomplicated MI. Nine of the 11 patients (82%) who developed TdP after MI in the absence of LQTS disease-causing mutations were found to be carriers of the K897T polymorphism on *KCNH2*, with a minor allele frequency of 0.41. The remaining 2 cases were homozygous for the major allele. Among the 133 uncomplicated MI controls, 86 (65%) were homozygous for the WT K allele, 40 (30%) were heterozygous, and 7 (5%) were homozygous for T897, with a minor allele frequency of 0.20. The Hardy-Weinberg equilibrium was respected in controls ($X^2 = 0.66$), while in cases there was an overrepresentation of the 897T polymorphism ($X^2 = 5.27$; $P < .05$). Thus, 35% of the MI controls were carriers of the K897T polymorphism, as already described for Caucasian control populations, but this percentage was 82% in patients with TdP following MI ($P = .0035$; Figure 4). Importantly, *KCNH2*-K897T was associated with an 8-fold increased risk of developing TdP during the healing phase of MI (95% confidence interval = 2–40; $P = .0035$).

NOS1AP (rs10494366, rs16847548, and rs4657139) genotyping

The 3 *NOS1AP* SNPs previously associated with QT interval and arrhythmic risk in LQTS^{9,13} showed no association with TdP risk in the subacute phase of MI. Specifically, *NOS1AP*-rs10494366 polymorphism was present in 70% of the cases and 65% of the controls ($P = 1$), *NOS1AP*-rs16847548 polymorphism was present in 40% of the cases and 41% of the controls ($P = 1$), and *NOS1AP*-rs4657139 polymorphism was present in 70% of the cases and 60% of the controls ($P = 0.74$). The Hardy-Weinberg equilibrium was respected in both cases and controls for the 3 SNPs.

KCNH2-K897T and QT interval

Correlation between the *KCNH2*-K897T polymorphism and QTc was investigated in all TdP cases devoid of LQTS mutations as well as in ethnically matched controls with uncomplicated MI in which an ECG was available at day 5 after MI (9 cases and 52 controls).

The KK genotype was associated in our controls with a longer QTc than in carriers of the minor T allele (443 ± 44 ms vs 417 ± 28 ms; $P = .03$). In contrast, the trend in our TdP cases was exactly the opposite. The only 2 cases with the KK genotype had a QTc of 487 ± 3 ms, while the remaining 7 cases that are carriers of the minor T allele had a longer QTc (553 ± 77 ms). Limiting our analysis to cases with an ECG available at day 5 after MI and a QTc of ≥ 480 ms, all those with the T allele developed TdP (7 of 7, 100%), but only 2 of the 9 with the KK genotype (22%) ($P = .0032$; Figure 5). In contrast, whenever the QTc was below 480 ms, there seemed to be no risk of developing TdP, independent of genotype.

The best approach for detecting a risk for TdP during the healing phase of MI would appear to be a combination of QTc and K897T. Indeed, QTc stratified at ≥ 480 ms has a 100% negative predictive value, whereas in patients with a QTc of ≥ 480 ms, K897T has a 100% positive predictive value.

Discussion

The main finding of the present study is that a common genetic variant, the *KCNH2*-K897T polymorphism, previously shown to increase arrhythmic risk in patients with the congenital

LQTS, is associated with a much higher probability of developing TdP during the first few days following an acute MI.

The impact of a genetic predisposition toward the occurrence of TdP following an acute MI is compounded by the fact that almost 85% of the patients with this arrhythmic complication carried either the polymorphism (69%) or an actual LQTS disease-causing mutation (15%) that had remained asymptomatic and unrecognized.

Our data provide clear evidence in support of the hypothesis that modifiers of arrhythmic risk in LQTS may favor the occurrence of life-threatening arrhythmias in a much more prevalent cardiac disease such as MI. This strengthens the unique role of LQTS as a useful paradigm to understand life-threatening cardiac arrhythmias and sudden death.

Mutations on LQTS genes and arrhythmic risk during and after MI

Ventricular fibrillation (VF) is the most common arrhythmia underlying SCD.²² In 80% of the cases, SCD occurs in the context of myocardial ischemia, but our ability to predict who will be at risk for life-threatening arrhythmias during or after an MI is very limited. Strong data support the role for heritable factors in SCD risk,²⁻⁵ but few data are available relative to the genetic determinants of SCD risk associated with MI.^{6,23}

Mutations in *SCN5A* resulting in loss of function would be expected to amplify the effects of acute ischemia and thus predispose to life-threatening arrhythmias during the hyperacute phase of ST-elevation MI. Supporting this view is our recent finding that in a cohort of 19 patients with primary VF during the hyperacute phase of MI, the only patient who actually developed an arrhythmic storm of recurrent VF carried the *SCN5A*-G400A loss-of-function mutation.²³

Another form of polymorphic ventricular tachycardia related to MI is the typical pause-dependent TdP, following excessive QT prolongation, which occurs during the healing phase of MI in the absence of ongoing ischemia.¹⁰ Infarct-related TdP is uncommon¹⁰ but potentially lethal and thus far its underlying mechanism has not been explored.

In our cohort of 13 cases, an LQTS mutation was identified in 2 cases. In both cases, personal and family history was negative for syncope or SCD at the time of presentation.

These findings demonstrate that LQTS mutations, subclinical throughout the patient's life, but capable of modestly reducing repolarization reserve, can account for approximately 15% of the cases of pause-dependent TdP observed in our cohort during the subacute phase of MI.

***KCNH2*-K897T modifies arrhythmic risk in LQTS and in the subacute phase of MI**

KCNH2-K897T is a very common polymorphism, present in approximately 30% of the Caucasian controls, previously reported to be a genetic modifier of LQTS.^{11,12} In 2005, we reported that this polymorphism was able to unmask an otherwise subclinical form of LQTS, caused by the *KCNH2*-A1116V mutation.¹¹ The electrophysiological characterization showed that the coexpression of A1116V and K897T led to a strikingly greater reduction of I_{Kr} compared with that produced by each individual allele, explaining the severe phenotype observed in the proband with both A1116V and K897T.¹¹

More recently, we provided further evidence of the important role of K897T in modifying the arrhythmic risk in another LQT2 family.¹² In this case, the mother and a daughter affected by a very mild form of the disease carried a nonsense mutation on *KCNH2* predicting a stop codon (P926AfsX14).¹² The first daughter, carrying the same mutation as

well as the *KCNH2*-K897T polymorphism on the opposite allele inherited from the father, had very prolonged QT and incessant ventricular tachycardia in utero and died suddenly 2 days after birth. The mother's second pregnancy ended in the 9th week, and once again molecular screening revealed the coexistence of the nonsense mutation and K897T polymorphism.¹² Coexpression of either K897T or the P926AfsX14 nonsense mutation individually with WT resulted in no significant difference in current density compared with WT alone. In contrast, coexpression of K897T with P926AfsX14 severely reduced tail current density by 61%. The functional study confirmed our original finding¹¹ that the common polymorphism K897T can markedly accentuate the loss of function of mildly defective *HERG* channels.¹²

The evidence that the *KCNH2*-K897T polymorphism can reduce repolarization reserve and act as modifier of the arrhythmic risk in the presence of an enabling substrate (ie, an *HERG* mutation) prompted us to hypothesize that the *KCNH2*-K897T polymorphism could also be implicated in the occurrence of TdP in those patients with a marked QT prolongation during the healing phase of an MI. The subacute phase of MI is known to be associated with mild QT prolongation,¹⁰ but little is known about the electrical remodeling of the normal myocardium that occurs during the post-MI period. A reduction in net repolarizing current underlies the QT prolongation observed in the subacute stage of MI. The K897T polymorphism could interact with the post-MI substrate to exacerbate the reduction in repolarization reserve amplifying the degree of QT prolongation and leading to TdP. The appearance of progressively more negative T waves suggests that the action potentials on the epicardial surface of the heart are preferentially prolonged.²⁴ Additional studies are needed to identify the basis for this interaction.

A possible explanation for these results is suggested by the report of Gentile et al.²⁵ In the setting of ischemia, Akt protein kinase activation could lead to Akt-mediated phosphorylation of the phosphorylation site created by T897 and thereby to loss of function of I_{K_r} , causing QT prolongation and TdP. Catecholamine-mediated protein kinase A (PKA) activation during ischemia may interact with the Akt pathway to further accentuate the phosphorylation of *HERG*, causing further loss of function of I_{K_r} . This reduction in repolarization reserve, coupled with the reduced levels of outward currents caused by post-MI ion channel remodeling, may create the substrate for the development of TdP. In our uncomplicated post-MI controls, another factor clearly intervenes to prevent QTc prolongation and to generate a slight abbreviation of QTc. Additional studies are needed to elucidate the basis for this interaction. One possibility is that there is a predominance of hormones that signal through PI3K activation, which are predicted to turn on 897K and turn off 897T, thus causing augmentation of I_{K_r} , abbreviating the ventricular action potential and thereby QTc. In the absence of ischemia (absence of Akt activation), the predominant effect may likewise be to turn off T897, leading to a gain of function of I_{K_r} and QT abbreviation.^{26,27} The association of KT/TT phenotype with shorter QTc observed in our controls is consistent with the results of previously reported genome-wide association studies.²⁸⁻³¹ This hypothesis may also explain the association of the post-MI ECG phenotype (Figure 1) with stress-induced cardiomyopathy and the development of TdP, as seen in Takotsubo cardiomyopathy.³²

Indeed, while the K897T polymorphism was present in 35% of the uncomplicated MI cases (approximately the same percentage as in Caucasian control populations), it was strikingly more frequent (82%) in patients with TdP post-MI. The K897T polymorphism was associated with an 8-fold increased risk of developing TdP during the healing phase of MI ($P = .0035$), and this risk further increased in those patients with a QTc of ≥ 480 ms.

The combined use of QTc and K897T appears to be a reasonable approach for the identification of patients at risk of developing TdP during the healing phase of MI.

Our results suggest that genetic modifiers of the QT interval that may not be phenotypically expressed, or that may contribute to QT-interval shortening, at baseline, in the healing phase of an MI may interact with the mechanisms underlying repolarization reserve to prolong the QT interval.

NOS1AP does not act as modifier of the arrhythmic risk in MI

Different NOS1AP polymorphisms have been associated with QT interval in the general population^{31,33} and very recently 3 of them have been shown to act as genetic modifiers in LQTS.^{9,13} These new findings prompted us to verify the possible role of these SNPs in favoring TdP in the subacute phase of MI. In this specific context, rs10494366, rs16847548, and rs4657139 did not show any favoring role, as they had no association with the risk of VF during the acute phase of MI,^{6,34} despite being associated with SCD risk.³⁵

Clinical implications

A subclinical form of LQTS should be suspected in any patient with an acute MI who develops marked QT prolongation with and without TdP in the absence of hypokalemia, hypomagnesemia, and/or QT-prolonging drugs. Family and personal history should be carefully collected, and genetic screening should be performed to exclude the presence of an LQTS disease-causing mutation. Such an evaluation is important, not only for the patient but also for family members who could be at risk of developing life-threatening arrhythmias if not identified and treated. The occurrence of malignant arrhythmias in ischemic heart disease, and probably in heart failure as well, may reflect an underlying genetic predisposition.

Study limitations

There are 2 major limitations with this study, one dealing with the absence of a second population for validation and the other dealing with the impossibility of using a genome-wide association study approach to search for all genes potentially influencing TdP risk during the subacute phase of MI. Both limitations are related to the fact that the specific phenotype under study is an uncommon one, which makes it impossible to reach adequate numbers for a genome-wide association study. Our study was not designed to give a prevalence of TdP during the subacute phase of MI, and our population was collected over a period of 10 years, making it almost impossible to gather a second population for validation. However, the phenotype under study, despite being associated with MI, has clinical manifestations similar to LQTS,³⁶ and indeed it was previously labeled as a variant of the acquired LQTS.¹⁰ Therefore, we used a very solid and evidence-based approach, searching for mutations on LQTS genes and for all polymorphisms described so far as modifiers of arrhythmic risk in LQTS. Indeed, we observed that in 15% of the cases a latent form of LQTS was identified and in 82% of the remaining cases the *KCNH2*-K897T polymorphism was present.

Conclusion

A genetic substrate underlies an excessive QT prolongation and life-threatening TdP in the sub-acute phase of myocardial infarction. Indeed, in 15% of the cases we identified a previously unknown LQTS disease-causing mutation, and in 82% of the remaining cases, the common K897T polymorphism was present and associated with an 8-fold increased risk. Our findings support the concept that the electrical remodeling associated with the healing phase of MI may unmask a genetic substrate predisposing to time-limited development of

life-threatening arrhythmias. Additionally, these data represent the proof of concept that modifiers of the arrhythmic risk in a monogenic disease as the LQTS, may increase risk also in much more prevalent diseases, such as myocardial infarction.

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References

1. Myerburg, R.J.; Castellano, A. Cardiac arrest and sudden cardiac death. In: Libby, P.; Bonow, R.O.; Mann, D.L., et al., editors. *Braunwald's Heart Disease: A Text of Cardiovascular Medicine*. 8. Philadelphia, PA: Elsevier Saunders; 2007. p. 933-974.
2. Friedlander Y, Siscovick DS, Weinmann S, et al. Family history as a risk factor for primary cardiac arrest. *Circulation*. 1998; 97:155–160. [PubMed: 9445167]
3. Jouven X, Desnos M, Guerot C, Ducimetiere P. Predicting sudden death in the population: the Paris Prospective Study I. *Circulation*. 1999; 99:1978–1983. [PubMed: 10209001]
4. Dekker LR, Bezzina CR, Henriques JP, et al. Familial sudden death is an important risk factor for primary ventricular fibrillation: a case-control study in acute myocardial infarction patients. *Circulation*. 2006; 114:1140–1145. [PubMed: 16940195]
5. Kaikkonen KS, Kortelainen ML, Linna E, Huikuri HV. Family history and the risk of sudden cardiac death as a manifestation of an acute coronary event. *Circulation*. 2006; 114:1462–1467. [PubMed: 17000909]
6. Bezzina CR, Pazoki R, Bardai A, et al. Genome-wide association study identifies a susceptibility locus at 21q21 for ventricular fibrillation in acute myocardial infarction. *Nat Genet*. 2010; 42:688–691. [PubMed: 20622880]
7. Schwartz PJ, Wolf S. QT interval prolongation as predictor of sudden death in patients with myocardial infarction. *Circulation*. 1978; 57:1074–1077. [PubMed: 639227]
8. Chugh SS, Reinier K, Singh T, et al. Determinants of prolonged QT interval and their contribution to sudden death risk in coronary artery disease: the Oregon Sudden Unexpected Death Study. *Circulation*. 2009; 119:663–670. [PubMed: 19171855]
9. Crotti L, Monti MC, Insolia R, et al. *NOS1AP* is a genetic modifier of the long QT syndrome. *Circulation*. 2009; 120:1657–1663. [PubMed: 19822806]
10. Halkin A, Roth A, Lurie I, Fish R, Belhassen B, Viskin S. Pause-dependent torsade de pointes following acute myocardial infarction: a variant of the acquired long QT syndrome. *J Am Coll Cardiol*. 2001; 38:1168–1174. [PubMed: 11583899]
11. Crotti L, Lundquist AL, Insolia R, et al. *KCNH2-K897T* is a genetic modifier of latent congenital long-QT syndrome. *Circulation*. 2005; 112:1251–1258. [PubMed: 16116052]
12. Nof E, Cordeiro JM, Perez GJ, et al. A common single nucleotide polymorphism can exacerbate long QT type 2 syndrome leading to sudden infant death. *Circ Cardiovasc Genet*. 2010; 3:199–206. [PubMed: 20181576]
13. Tomas M, Napolitano C, De Giuli L, et al. Polymorphisms in the *NOS1AP* gene modulate QT interval duration and risk of arrhythmias in the long QT syndrome. *J Am Coll Cardiol*. 2010; 55:2745–2752. [PubMed: 20538168]
14. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol*. 2009; 169:505–514. [PubMed: 19126586]
15. Moss AJ, Zareba W, Kaufman ES, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. *Circulation*. 2002; 105:794–799. [PubMed: 11854117]

16. Schwartz PJ, Stramba-Badiale M, Crotti L, et al. Prevalence of the congenital long-QT syndrome. *Circulation*. 2009; 120:1761–1767. [PubMed: 19841298]
17. Hofman N, Tan HL, Alders M, Van Langen IM, Wilde AAM. Active cascade screening in primary inherited arrhythmia syndromes: does it lead to prophylactic treatment? *J Am Coll Cardiol*. 2010; 55:2570–2576. [PubMed: 20513597]
18. Schwartz PJ. Cascades or waterfalls, the cataracts of genetic screening are being opened on clinical cardiology. *J Am Coll Cardiol*. 2010; 55:2577–2579. [PubMed: 20513598]
19. Viskin S, Postema PG, Bhuiyan ZA, et al. The response of the QT interval to the brief tachycardia provoked by standing: a bedside test for diagnosing long QT syndrome. *J Am Coll Cardiol*. 2010; 55:1955–1961. [PubMed: 20116193]
20. Ko YL, Tai DY, Chen SA, Lee-Chen GJ, Chu CH, Lin MW. Linkage and mutation analysis in two Taiwanese families with long QT syndrome. *J Formos Med Assoc*. 2001; 100:767–771. [PubMed: 11802537]
21. Käb S, Crawford DC, Sinner MF, et al. 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. [PubMed: 22100668]
22. Huikuri HV, Castellanos A, Myerburg RJ. Sudden death due to cardiac arrhythmias. *N Engl J Med*. 2001; 345:1473–1482. [PubMed: 11794197]
23. Hu D, Viskin S, Oliva A, et al. Novel mutation in the *SCN5A* gene associated with arrhythmic storm development during acute myocardial infarction. *Heart Rhythm*. 2007; 4:1072–1080. [PubMed: 17675083]
24. Emori T, Antzelevitch C. Cellular basis for complex T waves and arrhythmic activity following combined I_{Kr} and I_{Ks} block. *J Cardiovasc Electrophysiol*. 2001; 12:1369–1378. [PubMed: 11797994]
25. Gentile S, Martin N, Scappini E, Williams J, Erxleben C, Armstrong DL. The human ERG1 channel polymorphism, K897T, creates a phosphorylation site that inhibits channel activity. *Proc Natl Acad Sci U S A*. 2008; 105:14704–14708. [PubMed: 18791070]
26. Gentile S, Darden T, Erxleben C, et al. Rac GTPase signaling through the PP5 protein phosphatase. *Proc Natl Acad Sci U S A*. 2006; 103:5202–5206. [PubMed: 16549782]
27. Storey NM, Gentile S, Ullah H, et al. Rapid signaling at the plasma membrane by a nuclear receptor for thyroid hormone. *Proc Natl Acad Sci U S A*. 2006; 103:5197–5201. [PubMed: 16549781]
28. Bezzina CR, Verkerk AO, Busjahn A, et al. A common polymorphism in *KCNH2* (HERG) hastens cardiac repolarization. *Cardiovasc Res*. 2003; 59:27–36. [PubMed: 12829173]
29. Pfeufer A, Jalilzadeh S, Perz S, et al. Common variants in myocardial ion channel genes modify the QT interval in the general population: results from the KORA study. *Circ Res*. 2005; 96:693–701. [PubMed: 15746444]
30. Gouas L, Nicaud V, Berthet M, et al. Association of *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A* polymorphisms with QT_c interval length in a healthy population. *Eur J Hum Genet*. 2005; 13:1213–1222. [PubMed: 16132053]
31. Newton-Cheh C, Eijgelsheim M, Rice KM, et al. Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nat Genet*. 2009; 41:399–406. [PubMed: 19305408]
32. Madias C, Fitzgibbons TP, Sheikh-Ali AA, et al. Acquired long QT syndrome from stress cardiomyopathy is associated with ventricular arrhythmias and torsades de pointes. *Heart Rhythm*. 2011; 8:555–561. [PubMed: 21146632]
33. Arking DE, Pfeufer A, Post W, Kao WH, Newton-Cheh C, Ikeda M, West K, Kashuk C, Akyol M, Perz K, Jalilzadeh S, Illig T, Gieger C, Guo CY, Larson MG, Wichmann HE, Marbán E, O'Donnell CJ, Hirschhorn JN, Käb S, Spooner PM, Meitinger T, Chakravarti A. *Nat Genet*. 2006; 38:644–651. Epub 2006 Apr 30. [PubMed: 16648850]
34. Crotti L, De Ferrari GM, Insolia R, et al. *NOS1AP* and the risk of lethal arrhythmias in the acute phase of a first myocardial infarction. *Eur Heart J Suppl*. 2010; 31:1038. abstract.
35. Kao WH, Arking DE, Post W, et al. Genetic variations in nitric oxide synthase 1 adaptor protein are associated with sudden cardiac death in US white community-based populations. *Circulation*. 2009; 119:940–951. [PubMed: 19204306]

36. Schwartz PJ, Crotti L, Insolia R. Long QT syndrome: from genetics to management. *Circ Arrhythm Electrophysiol*. In press.

ABBREVIATIONS

ECG	electrocardiogram
HERG	human ether-a-go-go-related gene
I_{Kr}	delayed rectifier potassium current
LQTS	long QT syndrome
MI	myocardial infarction
QTc	QT interval corrected for heart rate
SCD	sudden cardiac death
SNP	single nucleotide polymorphism
TdP	torsades de pointes
VF	ventricular fibrillation
WT	wild type

Figure 1.

Electrocardiogram (ECG) tracings recorded from 2 post-myocardial infarction (MI) torsades de pointes (TdP) patients. **A:** Typical progression of ECG changes in a 47-year-old man following acute anterior infarction. Day 1: Streptokinase treatment—evident ST-segment elevation. QT interval corrected for heart rate (QTc) = 450 ms. Day 2: Partial resolution of ST-segment elevation; T-wave inversion evident in V4 and V5; QTc = 450 ms. Day 7: Inverted T waves develop throughout the precordium (QTc = 469 ms). Day 9: Deeply inverted T waves in V₁-V₆ (QTc = 540 ms). Day 11: Recurrent pause-dependent TdP despite beta-blocker and magnesium treatment. Day 60: The QT interval is normal (QTc = 400 ms). Modified from patient presented in Halkin et al.¹⁰ **B:** Prolonged QT intervals (QT = 600 ms; QTc = 820 ms) and pause-dependent TdP developing during day 4 of an acute inferior MI in an 88-year-old woman.

Figure 2. Clinical and molecular characteristics of the *HERG*-R744X mutation. **A:** Family pedigree. Circles denote female subjects, squares denote male subjects, and arrow denotes the proband. Affected members are depicted as filled symbols and those for which data are unavailable are shown in gray. **B:** Location of R744X mutation is indicated by using the predicted model structure of the Kv11.1 potassium ion channel. **C:** Chromatogram showing the substitution of thymine for a cytosine at position 2230 in *KCNH2*. WT = wild-type; N/A, not applicable.

Figure 3.

Molecular and electrophysiological characteristics of *SCN5A*-E446K mutation. **A:** Results of genomic DNA sequencing analysis of *SCN5A* exon 10. **B:** Location of E446K mutation is indicated by using the predicted transmembrane topology structure of the Nav1.5 channel. **C:** Alignment of the voltage-gated sodium channel α -subunit shows that E446 (in blue) is highly conserved among different species. **D-G:** Analysis of peak inward sodium currents (peak I_{Na}) for wild type (WT) and E446K. **D:** Representative sodium current traces recording from WT and mutants. Inward sodium currents were elicited by depolarizing pulses ranging from -100 to $+30$ mV in 5 -mV increments with a holding potential of -120 mV. **E:** Current-voltage relationship for WT (filled circles; $n = 33$) and E446K (open circles; $n = 22$). The current amplitude was significantly decreased for E446K when compared with WT at test potentials between -50 and -25 mV ($*P < .05$). **F:** Representative steady-state inactivation traces from both WT and mutant. **G:** Boltzmann distributions of voltage-dependent channel inactivation for WT (filled circles; $n = 17$) and E446K (open circles; $n = 20$). **H-I:** Analysis of late inward sodium currents (late I_{Na}) for WT and E446K. Representative sodium current traces recorded in the absence and presence of 25μ M TTX during 300 -ms depolarizations to -20 mV from -120 mV. Magnification of traces between 250 and 300 ms is shown in insets. Bar graph of relative late I_{Na} (% of peak I_{Na}) between WT and E446K groups.

Figure 4. Incidence of *KCNH2*-K897T polymorphism in post-myocardial infarction (MI) torsades de pointes (TdP) cases, uncomplicated MI controls, and Caucasian controls.

Figure 5.

QT interval corrected for heart rate (QTc) at the basal electrocardiogram measured at day 5 following myocardial infarction in cases and controls according to *KCNH2*-K897T genotype.