Table 1 Discovery and replication studies confirm three alleles on chromosomes 3 and 6 are associated with Brugada syndrome

Marker		rs11708996	rs10428132	rs9388451
Genome position (Build 37)		Chr. 3: 38633923	Chr. 3: 38777554	Chr. 6: 126090377
Closest gene(s)		*SCN5A*	*SCN10A*	*HEY2, NCOA7*
Risk allele		C	T	C
Protective allele		G	G	T
GWAS (312 cases and 1,115 controls; European)[a]	RAF[b]	0.23/0.15	0.69/0.41	0.65/0.50
	P	2.70×10^{-5}	6.79×10^{-26}	8.85×10^{-10}
	OR (CI)	1.64 (1.30–2.07)	3.00 (2.45–3.69)	1.83 (1.51–2.22)
Replication 1 (594 cases and 806 controls; European)[a]	RAF[b]	0.23/0.15	0.65/0.42	0.59/0.50
	P	1.10×10^{-7}	1.66×10^{-30}	2.10×10^{-5}
	OR (CI)	1.69 (1.39–2.04)	2.35 (2.03–2.72)	1.39 (1.19–1.61)
Replication 2 (208 cases and 1,016 controls; Japanese)[a]	RAF[b]	0.09/0.04	0.44/0.23	0.72/0.61
	P	5.63×10^{-5}	1.56×10^{-16}	6.70×10^{-6}
	OR (CI)	2.30 (1.53–3.45)	2.56 (2.05–3.19)	1.74 (1.37–2.21)
Meta-analysis	*P*	1.02×10^{-14}	1.01×10^{-68}	5.14×10^{-17}
	OR (CI)	1.73 (1.51–1.99)	2.55 (2.30–2.84)	1.58 (1.42–1.75)
	Heterogeneity	$Q = 2.17$	$Q = 3.68$	$Q = 5.68$
		$P = 0.338$	$P = 0.159$	$P = 0.058$
		$I^2 = 4\%$	$I^2 = 45\%$	$I^2 = 65\%$
Cases with symptoms[c] (416 cases and 2,937 controls; meta-analysis)[a]	*P*	6.88×10^{-8}	1.15×10^{-39}	5.01×10^{-8}
	OR (CI)	1.73 (1.42–2.12)	2.84 (2.43–3.32)	1.55 (1.32–1.81)

[a]Size of case and control sets; sample ancestry. [b]Risk allele frequency (RAF) in cases over controls. [c]Symptoms included ventricular tachycardia, ventricular fibrillation, syncope and near syncope.

(rs10428132, $P = 1.01 \times 10^{-68}$; rs11708996, $P = 1.02 \times 10^{-14}$; rs9388451, $P = 5.14 \times 10^{-17}$), with the corresponding effect sizes ranging from 1.58 to 2.55 (**Table 1**). Effect sizes were similar when the meta-analysis was restricted to symptomatic individuals, with the association for rs10428132 reaching genome-wide statistical significance (**Table 1**).

We next assessed the cumulative effect of the three loci on susceptibility to Brugada syndrome (**Fig. 2a** and **Supplementary Table 4**). We found that disease risk increased consistently with increasing numbers of carried risk alleles ($P_{trend} = 6.1 \times 10^{-81}$), with the estimated odds ratio (OR) reaching 21.5 in the presence of more than four risk alleles versus less than 2 (**Fig. 2b**). Under a multiplicative model, assuming a prevalence of 0.05% for Brugada syndrome[2] and using the ORs and allele frequencies reported in **Table 1**, the sibling relative risk attributable to the three lead SNPs was estimated at $\lambda_S = 1.4$, with the three loci accounting for 7% of the variance in disease susceptibility (Online Methods). The proportion of the phenotypic variance explained by only these three loci, as well as their cumulative effect, are unexpectedly large in comparison to previously reported effects for common genetic variation on susceptibility to common disease[25]. However, as 1.5% of the European population is expected to carry more than four risk alleles (**Supplementary Table 4**), these three polymorphisms are unlikely to by themselves explain the occurrence of Brugada syndrome and are only associated with a low absolute risk for this rare condition. Furthermore, the ORs reported here were calculated using data from case-control collections and thus may overestimate relative risks.

In subsequent analyses comparing SNP allele dosages in subsets of cases, we could not detect any consistent association of risk alleles with symptoms, *SCN5A* mutation status or Brugada syndrome type I ECG at baseline compared to after drug challenge (**Supplementary Table 5**).

Our strongest association (rs10428132) resides in intron 14 of the *SCN10A* gene, located adjacent to *SCN5A* on chromosome 3p21-22. The particular haplotype tagged by this SNP, which also contains a nonsynonymous variant affecting *SCN10A* (rs6795970, $r^2 = 0.97$ with rs10428132), has previously been associated with variability in PR interval and QRS duration in the general population[4–7]. *SCN10A*, which encodes the sodium channel isoform $Na_v1.8$, was originally reported to be highly expressed in nociceptive sensory neurons of the dorsal root ganglia and cranial sensory ganglia[26]. Besides being expressed in cardiac neurons[27], *SCN10A* was also shown in recent studies to be expressed in the working myocardium[4,28] and the specialized conduction system[7,29], indicating a possible role for $Na_v1.8$ in cardiac electrical function. An independent report implicated the rs6801957 SNP ($r^2 = 0.97$ with rs10428132) as a probable causal variant on the associated haplotype[30]. This SNP, which alters a highly conserved nucleotide within a consensus T-box–binding site, affects TBX5- or TBX3-mediated enhancer activity[30] and is thus expected to affect TBX5/TBX3-regulated expression of *SCN5A* and *SCN10A in vivo*[31]. Further studies are required to determine whether the effects of this SNP on conduction and Brugada syndrome are mediated through regulation of *SCN5A*, *SCN10A* or both. The other independent association at 3p21-22 (rs11708996) involved another haplotype previously associated with cardiac conduction[6,7]. For both haplotypes, the PR- and QRS-prolonging allele was associated with disease risk, providing support for the concept that the cardiomyocyte depolarization process has an important role in the pathogenesis of Brugada syndrome[12].

In contrast to both signals at the *SCN5A-SCN10A* locus, to our knowledge, the signal at 6q22 has never been detected by previous GWAS on ECG traits. The corresponding SNP tagged an LD block

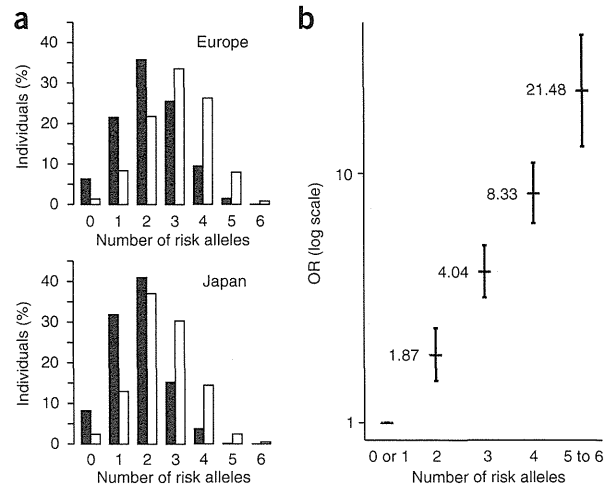

Figure 2 Cumulative effect of alleles at the three associated loci on susceptibility to Brugada syndrome. (**a**) Distribution of risk alleles among individuals with Brugada syndrome (white bars) and among control individuals (black bars) from Europe (top) and Japan (bottom). (**b**) ORs calculated according to the number of risk alleles carried. A meta-analysis was performed as described in the Online Methods, using individuals carrying no or one risk allele as the reference. Each black bar represents the log(OR) value (horizontal bar) and the 95% confidence interval (on the log scale; vertical bar).

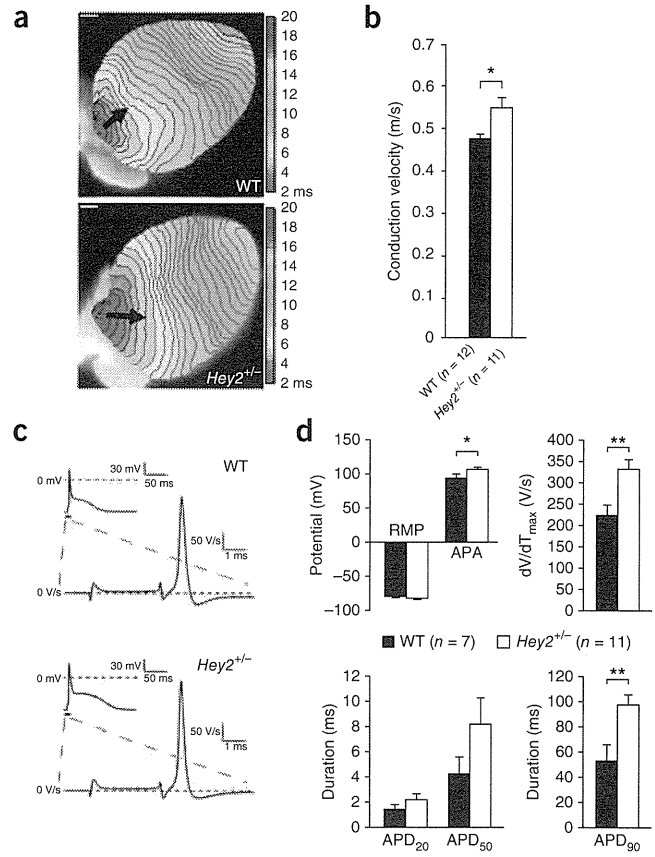

Figure 3 Increased conduction velocity and sodium channel availability in the RVOT of adult *Hey2*$^{+/-}$ mice. (**a**) Representative optical activation maps from isolated wild-type (WT; $n = 12$) and *Hey2*$^{+/-}$ ($n = 11$) hearts stimulated at the RVOT at a basic cycle length of 120 ms (scale bars, 1 mm). Arrows indicate conduction along the RVOT epicardium through the connection of similar isochrones. Isochrones (0.5-ms intervals) in the RVOT of *Hey2*$^{+/-}$ hearts are less crowded compared to wild-type isochromes, indicating faster conduction in the RVOT of *Hey2*$^{+/-}$ hearts (as indicated by increased arrow length). (**b**) On average, conduction velocity in the RVOT of *Hey2*$^{+/-}$ hearts was significantly increased compared to that in wild-type hears (*$P < 0.05$; Student's *t* test). Error bars, s.e.m. (**c**) Representative action potentials measured in adult wild-type (WT; $n = 7$) and *Hey2*$^{+/-}$ ($n = 11$) cardiomyocytes isolated from RVOT. (**d**) Average action potential characteristics measured at 4 Hz. RVOT cardiomyocytes from *Hey2*$^{+/-}$ mice showed a significant increase in maximal upstroke velocity (dV/dT_{max}, a measure of sodium channel availability and a determinant of conduction velocity) and action potential amplitude (APA), indicating increased sodium channel availability (*$P < 0.05$; **$P < 0.01$; Student's *t* test). Resting membrane potential (RMP), action potential duration at 20% and 50% repolarization (APD_{20} and APD_{50}, respectively) were not significantly different in wild-type and *Hey2*$^{+/-}$ mice; action potential duration at 90% repolarization (APD_{90}) was significantly increased in RVOT cardiomyocytes from *Hey2*$^{+/-}$ mice. Results are expressed as mean ± s.e.m.

that entirely encompasses the *HEY2* gene and extends into the first intron of *NCOA7* (also called *ERAP140*), which encodes a nuclear receptor coactivator that interacts with estrogen receptor α to modulate its activity[32]. *NCOA7* is predominantly expressed in the brain[32] and has not been implicated in cardiovascular function. In contrast, *HEY2* (also called *HESR2*, *HRT2* and *CHF1*) encodes a basic helix-loop-helix (bHLH) transcriptional repressor that is expressed in the cardiovascular system[8].

Studies we have conducted in *Hey2*-targeted mice provide strong support for the role of this gene in Brugada syndrome. In the developing mouse heart, *Hey2* expression is confined to the (subepicardial) compact myocardium of the ventricle[33]. *Hey2*-null mice exhibit a spectrum of developmental anomalies, including ventricular wall thinning, abnormal right ventricular morphology and postnatal cardiomyopathic changes[34–37]. The expression of *Gja5* (encoding Cx40), *Nppa* and *Tbx5*, normally enriched in the (subendocardial) trabecular component of the ventricle, is expanded into the compact myocardium in *Hey2*-deficient embryos[34,38,39]. Because such transmural heterogeneity in expression is similarly well established for $Na_v1.5$ (high expression in subendocardium, low expression in subepicardium)[40], loss of *Hey2* might also affect the transmural expression gradient of this ion channel implicated in Brugada syndrome[2,3]. Indeed, in hearts from homozygous *Hey2*-null embryos, we observed higher $Na_v1.5$ expression in the compact layer than in wild-type hearts, flattening the expression gradient of this channel (**Supplementary Fig. 8a**).

The functional consequences of Hey2 loss were investigated in adult heterozygous *Hey2* mice (*Hey2*$^{+/-}$), which have structurally normal hearts (**Supplementary Fig. 9**). *In vivo* surface ECG parameters were unchanged in *Hey2*$^{+/-}$ mice (**Supplementary Fig. 10**). However, conduction velocity was significantly increased in the right ventricular outflow tract (RVOT) of isolated *Hey2*$^{+/-}$ hearts (**Fig. 3a,b**), whereas conduction velocity was unaffected in the right and left ventricular free wall (**Supplementary Fig. 11**). Action potential upstroke velocity was increased in *Hey2*$^{+/-}$ myocytes isolated from the RVOT region (**Fig. 3c,d**), pointing to increased sodium channel function, despite undetectable changes in $Na_v1.5$ expression in adult hearts from *Hey2*$^{+/-}$ mice in immunohistochemistry analysis (**Supplementary Fig. 8b**). Furthermore, the prolonged repolarization parameters observed in these cells suggest an additional regulatory role for Hey2 in repolarizing currents (**Fig. 3d**). Future work should address whether the observed alterations in action potential characteristics and conduction are mediated through ion channel correlates, subtle structural heart alterations or both. Nevertheless, the preferential involvement of the RVOT is in line with ECG manifestations in right precordial leads and concurs with the observation that the RVOT is a common site of origin of ventricular arrhythmias in individuals with Brugada syndrome[41].

In conclusion, we have identified Hey2 as a transcriptional regulator of cardiac electrical function involved in the pathogenesis of Brugada syndrome. Furthermore, we provide new evidence that common variants, previously shown to modulate ECG conduction indices, also modulate susceptibility to a rare arrhythmia disorder. Most notably, this study demonstrates that the GWAS paradigm can be successfully applied to a rare disorder, previously considered monogenic, to identify common genetic variants with unexpectedly strong modifier effects.

URLs. Affymetrix Power Tools, http://www.affymetrix.com/partners_programs/programs/developer/tools/powertools.affx; GTOOL, http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html; R statistical package, http://www.r-project.org/; 1000 Genomes Project, http://www.1000genomes.org/; 1000 Genomes phase I integrated variant set release, http://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_integrated.html.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank L. Beekman, C. de Gier-de Vries, B. de Jonge and the Genomic Platform of Nantes (Biogenouest Genomics) for technical support. We are also grateful to the French Clinical Network against Inherited Cardiac Arrhythmias, which includes the University Hospitals of Nantes, Bordeaux, Rennes, Tours, Brest, Strasbourg, La Réunion, Angers and Montpellier. This study was funded by research grants from the Leducq Foundation (CVD-05; Alliance Against Sudden Cardiac Death), the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant-in-aid for the Project in Sado for Total Health, PROST), the French Ministry of Health (PHRC AOR04070, P040411 and PHRCI DGS2001/0248), INSERM (ATIP-Avenir program to R.R.) and the French Regional Council of Pays-de-la-Loire. This research was also supported by the Netherlands Heart Institute (grant 061.02 to C.A.R. and C.R.B.) and the Division for Earth and Life Sciences (ALW; project 836.09.003 to C.A.R.) with financial aid from the Netherlands Organization for Scientific Research (NWO). C.R.B. acknowledges support from the Netherlands Heart Foundation (NHS 2007B202 and 2009B066). J.B. was supported by a research grant from the European Society of Cardiology and the Netherlands Heart Institute (ICIN) and by the French-Dutch Academy through the Van Gogh program. E.S.-B. was supported by the Interdisziplinären Zentrums für Klinische Forschung (IZKF) of the University of Münster and the Collaborative Research Center SFB656. M.G. acknowledges support from the German Research Foundation (DFG-SFB 688 and TP A16). This manuscript is dedicated to the memory of Denis Escande, who founded the Leducq Foundation Network Alliance Against Sudden Cardiac Death.

AUTHOR CONTRIBUTIONS

C.R.B., J.-J.S. and R.R. designed the study. Y.M. and J.-B.G. evaluated all ECGs. C.D. coordinated the statistical analyses, which C.D., F.S., P.L. and E.C. carried out. F.G., A.D., S.L. and E.C. performed genotyping for the GWAS. J.B., J.V., V. Portero and K.H. carried out genotyping in the validation sets. A.A.W., H.L.T., H.L.M., V. Probst, F.K., S. Bézieau, S.C., S.K., B.M.B., E.S.-B., S.Z., L.C., P.J.S., F.D., M.T., C.A., S. Bartkowiak, P.G., V.F., A.L., D.M.R., P.W., E.R.B., R.B., J.T.-H., M.S.O., N.M., A.N., M.H., S.O., K.H., W.S. and T.A. recruited subjects and participated in clinical and molecular diagnostics. P.F., B.B., O.L., H.W., T.M. and N.E. provided controls. M.G., D.W. and C.W. provided the mice. C.A.R., A.O.V., B.J.B. and R.W. acquired and analyzed electrophysiological data. V.M.C., C.A.R. and R.W. acquired and analyzed protein expression data. C.R.B., J.B., C.A.R., C.D., J.-J.S., V.M.C., R.C. and R.R. interpreted the data. C.R.B., J.-J.S., V. Probst, D.M.R., A.A.W., S.K., E.S.-B., A.L. and R.R. obtained funding. C.R.B., J.B., C.D. and R.R. drafted the manuscript. All coauthors critically revised the manuscript for intellectual content. C.R.B. and R.R. led the study together.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Case and control samples. Individuals with Brugada syndrome, defined by the presence of a type 1 ECG[2], were recruited from 13 centers in Europe (Nantes, Paris, Amsterdam, Pavia, Copenhagen, Munich, Münster and London), the United States (Utica and Nashville) and Japan (Nagasaki, Shiga and Osaka). Only index cases were included from extended pedigrees. Appropriate medical ethical committee approval was obtained at each participating clinical center. Informed consent was available from all subjects. Clinical data (age at diagnostic ECG, *SCN5A* mutation status, symptoms and family history of sudden cardiac death) and ECGs were collected centrally and reviewed. A Brugada syndrome type I ECG pattern was defined on the basis of the criteria drawn out at the Second Consensus Conference on Brugada Syndrome[2], namely, a coved type ST elevation at baseline or after a drug challenge test, in one or more leads in the right precordial leads, including the third and fourth intercostal space. Drug challenge tests were performed according to consensus criteria[2]. Control subjects were drawn from the D.E.S.I.R. cohort[18] for the GWAS and the European replication set and were drawn from the Sado study[42] for the Japanese replication set. No statistical method was used to predetermine sample size.

GWAS genotyping. SNP genotyping was performed on population-optimized Affymetrix Axiom Genome-Wide CEU 1 array plates following the standard manufacturer's protocol. Each array contains 567,097 SNPs. Fluorescence intensities were quantified using the Affymetrix GeneTitan Multi-Channel Instrument, and primary analysis was conducted with Affymetrix Power Tools following the manufacturer's recommendations (see URLs). Genotype calling, a two-dimensional clustering analysis, was performed using the 'apt' program. Individuals with genotype call rate of lower than 97% were removed, as were those with fewer than 10,000 markers reporting a heterozygous state (the threshold was determined after visual inspection). Monomorphic SNPs were excluded, as were those with minor allele frequency (MAF) of <10% (n = 175,153), a call rate of <95% (n = 19,986) or Hardy-Weinberg disequilibrium in controls (n = 2,054 with $P < 1 \times 10^{-4}$ when testing for Hardy-Weinberg equilibrium). Note that Hardy-Weinberg disequilibrium was also tested in demographically homogenous cases to identify very large deviations ($P < 1 \times 10^{-7}$). Additional SNPs were excluded for batch effect: such SNPs were defined as those with significant differences in allele frequency in one plate versus all others within cases and within controls only (n = 68) or with unexplained large differences observed in controls versus the 1000 Genomes Project European (non-Finnish) population (n = 9,686).

Demographic analyses. The ancestry of individuals was assessed using a multidimensional scaling technique, as implemented in PLINK[43]. SNPs were selected for short-range LD independence. Pruning was performed using a two-step procedure to accommodate longer range LD (this is particularly important, as the Axiom Human array is enriched in SNPs in the human leukocyte antigen (HLA) region). In a first step, we applied the threshold $r^2 < 0.2$ within a 20-kb LD block or within 50 SNPs. In a second step, we applied the same threshold within a 10-Mb distance or within 100 SNPs on the pruned data set. We then created an identity-by-state (IBS) matrix including all individuals and applied the multidimensional scaling method (–mds option in PLINK) to retrieve the first five components. Three matrices were estimated using our cases and controls together with all 1000 Genomes Project populations (IC) and all European (except Finnish) populations (E). At each level, we excluded outliers on the first two components using an expectation-maximization-fitted Gaussian mixture clustering method[44] implemented in the R package M-CLUST, assuming either three (for IC) or two (for E) clusters and noise. Outlier position was assigned using nearest-neighbor-based classification[45] (NNclust in R package PrabClus). Outliers were excluded from the analysis, as previously done in GWAS[46].

GWAS. Using the clustering algorithm described above, we defined two homogenous groups (A and B). To carry out the genome-wide analysis, each SNP was tested within groups A and B separately, using logistic regression and assuming an additive genetic model with adjustment for the first five components retrieved. No additional covariates were added, as advised[47]. Instead, the results from groups A and B were combined into a meta-analysis using an inverse normal strategy[48], whereby the summary P values for each test (and effect direction) are combined into a signed z score that, properly weighted, yields $N(\mu = 0, \sigma^2 = 1)$. Because the number of controls exceeded by far the number of cases in all studies, we used the effective sample size (weighting studies A and B) using METAL software as advised[49]. In addition, we performed a second genome-wide analysis on a homogenous sample of 254 cases and 806 controls of apparent French origin (largest geographically homogenous sample; **Supplementary Fig. 5**).

Concordance rate between Axiom and 1000 Genomes Project data. We genotyped 95 HapMap individuals on Affymetrix Axiom Genome-Wide CEU 1 arrays using the same process as described above. We could retrieve the genotypes of 58 of these 95 individuals from the 1000 Genomes Project database. The concordance rate was tested using PLINK (merging mode 7, which compares the common non-missing genotypes). The concordance rate was 99.4% over a total of 20,853,552 genotypes and 100% over the 174 genotypes corresponding to the 3 associated SNPs.

Genome-wide imputation analysis. Genotyped SNPs in cases and controls were phased using the SHAPE-IT (v.1) program[50]. Imputation of 6.1 million frequent SNPs (MAF > 0.05 in Europeans) was carried out using IMPUTE v2 (ref. 51). Chromosome regions were split in chunks of approximately 7 Mb. The reference panel was Phase I integrated variant set release (v3) in NCBI Build 37 (hg19) coordinates (see URLs). For each chromosomal chunk, a set of genetically matched panel individuals was selected, according to the last strategy used by IMPUTE[52]. Imputed SNPs were combined with SNPs extracted from the 1000 Genomes Project data set under the IMPUTE format. We merged both data sets using GTOOL. We applied a logistic regression (additive model) as implemented in SNPTEST[45] (options -frequentist 1 and -score), using the first five components as covariates. Individuals from the 1000 Genomes Project data set were added for all SNPs either genotyped or imputed.

Post-analysis quality control. For each significantly associated SNP in a region (called here lead SNP), we visually inspected the cluster graph (**Supplementary Fig. 12a**) and the association results of SNPs in LD (locus plot).

Enrichment analysis. We tested enrichment in moderately associated SNPs grouped by distinctive properties. The first group included SNPs that have been reported to be associated with ECG traits in previous GWAS. We used the list published in ref. 53 after removal of redundant SNPs (one SNP was selected randomly for each group of SNPs with $r^2 > 0.2$) and exclusion of SNPs located in the same haplotype block as rs10428132. The second list comprised every SNP located within 1-Mb intervals (500 kb upstream and 500 kb downstream) centered on the susceptibility genes for Brugada syndrome listed in ref. 13. We removed the *SCN5A* gene, as it would have disproportionately inflated the overall statistics. Quantile-quantile plots were first visually inspected to check for enrichment. The significance of enrichment suggested by the quantile-quantile plot for the first group (ECG-associated SNPs) was formally tested by a simple Fisher combination test.

Replication genotyping. The three significantly associated SNPs from the GWAS stage (rs10428132, rs9388451 and rs11708996) were typed for the replication steps by TaqMan SNP Genotyping assays (Applied Biosystems) according to the manufacturer's protocol on a LightCycler 480 Real-Time PCR System (Roche) or an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Assays C__26860683_10, C___2824356_10 and C__44417794_10 were functionally tested by Applied Biosystems for the SNPs rs10428132, rs9388451 and rs11708996, respectively. Reaction conditions included an initial denaturation step at 95 °C for 10 min, followed by 50 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 30 s. Data were analyzed with LightCycler 480 software (release 1.5.0 SP4; **Supplementary Fig. 12b**) or by ABI 7900HT Sequence Detection Systems software (**Supplementary Fig. 12c**). Outliers were excluded from the analysis. After the first round of genotyping, two samples per SNP genotype cluster were sequenced to verify genotype (**Supplementary Fig. 12b**). Furthermore, 15 samples that were previously genotyped on Affymetrix Axiom Genome-Wide CEU 1 arrays were used as control samples: the genotypes obtained by TaqMan assays were perfectly concordant with those generated with Axiom array technology.

SNP imputation for the control population used in the European replication. The genotypes of the three SNPs tested in replication were imputed using IMPUTE software for 806 D.E.S.I.R. individuals on the basis of genotype data sets previously generated on Illumina Human CNV370-Duo arrays[24,54]. We selected a threshold of 0.9 for genotype calling, meaning that the genotypes whose posterior probability exceeded this threshold were called, whereas those where this probability was below the threshold were set as unknown. We genotyped 49 individuals on both Affymetrix and Illumina arrays. Once hard calls were obtained using GTOOL (see URLs) with -Gen mode and –threshold option set to 0.9, concordance rates were calculated using PLINK (merging mode 7). Perfect concordance was observed between genotyped and imputed SNPs (100% concordance rate for 144 genotypes).

Replications and meta-analyses. The case-control replication study was performed using a logistic regression method that accounts for genotype calling uncertainty. This method, based on missing data theory, allows the unbiased estimation of ORs and confidence intervals and is implemented in SNPTEST (options–method ml). Pooled ORs were obtained by averaging the ORs from all stages (GWAS and European and Japanese replications) and weighted by the inverse of the variance. Heterogeneity was tested using the Cochran's Q test and was also measured using Higgins' index[55]. We generated genetic scores for individuals on the basis of an allelic scoring system involving our three SNPs. These scores were created either through the number of at-risk alleles for the European discovery and Japanese replication populations or the risk allele dosage in the European replication population. Risk allele dosages from the European replication population were collapsed using dosage. The distribution of imputed dosage is shown in **Supplementary Figure 13a**. Results were similar between imputed dosage ($\beta = 0.62921$, $\sigma = 0.05427$, $P = 4.43 \times 10^{-31}$) and collapsed imputed dosage ($\beta = 0.61778$, $\sigma = 0.05386$, $P = 1.88 \times 10^{-30}$). Moreover, we compared the genetic scores obtained with genotyped versus imputed SNPs for 49 individuals who were genotyped on Axiom Genome-Wide CEU 1 arrays and imputed with the European replication population and observed high correlation between methods (**Supplementary Fig. 13b**). Finally, we tested whether a non-additive model (recessive or dominant) might be a better fit for each genome-wide significant SNP. A heterozygote effect was added to the logistic regression analysis along with the linear effect (effect of the number of alternative alleles) in each study and in a meta-analysis. We did not detect any consistent deviation from the additive model (**Supplementary Table 6**).

Estimation of the genetic score effect by multiple imputation. Despite this high concordance, we chose to estimate genotype score risk within the Multiple Imputation framework[56]. Ten data sets were created where each uncertain genotype was replaced by a value simulated under the probability distribution obtained through genetic imputation (IMPUTE output consisting of P(AA), P(AB) and P(BB)). The β value and variance were obtained using standard procedures[57]. We let m be the number of simulations (called replicates). For each simulation, we carried out a logistic regression (either on score as an ordinal value or on each score versus baseline). The Multiple Imputation effect estimation was calculated as follows:

$$\bar{\beta}_{\mathrm{MI}} = \frac{1}{m}\sum_{j=1}^{m}\beta_j$$

The variance was calculated as the sum of within-replicate and between-replicate variances

$$\sigma^2_{\mathrm{MI}} = \frac{1}{m}\bar{\sigma}^2_j + \frac{1}{m-1}\sum_{j=1}^{m}(\bar{\beta}_{\mathrm{MI}} - \beta_j)^2$$

Confidence intervals were retrieved using the 95% quantile of a Student distribution with a number of degrees of freedom, which is a function of the two components of the variance. We used the 'glm' function of the R statistical package (see URLs) to perform logistic regression. R was used to create complete data sets from IMPUTE output.

Calculation of sibling relative risk and liability-scale variance. Assuming a multiplicative model (within and between variants), the contribution to the sibling relative risk of a set of N SNPs was calculated using the following formula

$$\lambda_s = \prod_{j=1}^{N}\left[1 + \frac{p_j(1-p_j)(\mathrm{OR}_j - 1)^2}{2[(1-p_j) + p_j\mathrm{OR}_j]^2}\right]^2$$

where p_j and OR_j denote the RAF and corresponding allelic OR at the jth SNP[58]. Assuming disease prevalence K, the liability-scale variance[59] explained by these SNPs was calculated as follows:

$$h^2_L = \frac{2[T - T_1\sqrt{1-(T^2 - T_1^2)}(1 - T/\omega)]}{\omega + T_1^2(\omega - T)}$$

In the expression, $T = \varphi^{-1}(1-K)$, $T_1 = \varphi^{-1}(1-\lambda_S K)$ and $\omega = z/K$, where z is the height of the standard Gaussian density at T.

Surface ECG analysis. Mice (male and female, aged 3–5 months) were anesthetized using isoflurane inhalation (0.8–1.0 volume percentage in oxygen), and surface ECGs were recorded from subcutaneous 23-gauge needle electrodes attached to each limb using the Powerlab acquisition system (ADInstruments). ECG traces were signal averaged and analyzed for heart rate (RR interval) and for PR-interval, QRS-interval and QT-interval duration using LabChart7Pro software (ADInstruments). QT intervals were corrected for heart rate using the formula $\mathrm{QTc} = \mathrm{QT}/\sqrt{\mathrm{RR}/100}$ (RR in ms).

Optical mapping in isolated hearts. Mice were anesthetized by an intraperitoneal injection of pentobarbital, after which the heart was excised, cannulated, incubated in 10 ml Tyrode's solution containing 15 μM Di-4 ANEPPS and subsequently placed in an optical mapping setup. Hearts were perfused at 37 °C with Tyrode's solution (128 mM NaCl, 4.7 mM KCl, 1.45 mM $CaCl_2$, 0.6 mM $MgCl_2$, 27 mM $NaHCO_3$, 0.4 mM NaH_2PO_4 and 11 mM glucose (pH maintained at 7.4 by equilibration with a mixture of 95% O_2 and 5% CO_2)) containing blebbistatin to prevent movement artifacts. Excitation light was provided by a 5-W power LED (filtered 510 ± 20 nm). Fluorescence (filtered for >610 nm) was transmitted through a tandem lens system on a CMOS sensor (100 × 100 elements; MICAM Ultima). Hearts were paced at a basic cycle length (BCL) of 120 ms (twice the diastolic stimulation threshold) from the center of the right or left ventricle epicardial surface or from the RVOT epicardial surface. Optical action potentials were analyzed with custom software. Local activation was defined as the maximum positive slope of the action potential, and local activation times were used to construct ventricular activation maps. To calculate conduction velocity in longitudinal and transversal directions in the right and left ventricles, the difference in activation time between at least three consecutive electrode terminals separated by a known distance located parallel (longitudinal) or perpendicular (transversal) to the direction of propagation was measured. The direction of propagation was determined using isochronal maps. For c velocity measurements in the RVOT, transverse fiber direction was defined as the slowest conduction velocity.

Action potential measurements in isolated cardiomyocytes. Cardiomyocytes were isolated by enzymatic dissociation as previously described[60]. After perfusion of the heart with enzyme solution, the RVOT area at the base of the right ventricle just below the truncus pulmonalis was excised and used during the subsequent isolation process[43]. Quiescent rod-shaped cross-striated cells with a smooth surface were selected for measurements. Action potentials were recorded with the amphotericin-B-perforated patch-clamp using an Axopatch 200B Clamp amplifier (Molecular Devices Corporation). Signals were filtered (low pass, 10 kHz) and digitized (40 kHz), and action potentials were corrected for the estimated change in liquid junction potential. Action potentials were measured at 36 ± 0.2 °C using a modified Tyrode's solution containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.5 mM glucose and 5.0 mM HEPES, pH 7.4 (NaOH). Pipettes (1.5–2.5 MΩ) were filled with solution containing 125 mM potassium gluconate, 20 mM KCl, 10 mM NaCl, 0.22 mM amphotericin-B and 10 mM HEPES, pH 7.2 (KOH). Action potentials were elicited at 4 Hz by 3 ms, 1.2× threshold current pulses

through the patch pipette. We analyzed resting membrane potential (RMP), action potential amplitude (APA), maximal upstroke velocity (dV/dt_{max}) and action potential duration at 20, 50 and 90% repolarization (APD_{20}, APD_{50} and APD_{90}, respectively). Data from ten consecutive action potentials were averaged. Results are expressed as mean ± s.e.m. Two sets of data were considered significantly different if the *P* value of the unpaired Student's *t* test with Bonferroni correction was <0.05.

Other methods. Procedures for immunohistochemistry hybridization on mouse heart sections were performed as described previously[40].

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Identification of a *KCNQ1* Polymorphism Acting as a Protective Modifier Against Arrhythmic Risk in Long-QT Syndrome

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Background—Long-QT syndrome (LQTS) is characterized by such striking clinical heterogeneity that, even among family members carrying the same mutation, clinical outcome can range between sudden death and no symptoms. We investigated the role of genetic variants as modifiers of risk for cardiac events in patients with LQTS.

Methods and Results—In a matched case–control study including 112 patient duos with LQTS from France, Italy, and Japan, 25 polymorphisms were genotyped based on either their association with QTc duration in healthy populations or on their role in adrenergic responses. The duos were composed of 2 relatives harboring the same heterozygous *KCNQ1* or *KCNH2* mutation: 1 with cardiac events and 1 asymptomatic and untreated. The findings were then validated in 2 independent founder populations totaling 174 symptomatic and 162 asymptomatic patients with LQTS, and a meta-analysis was performed. The *KCNQ1* rs2074238 T-allele was significantly associated with a decreased risk of symptoms 0.34 (0.19–0.61; $P<0.0002$) and with shorter QTc ($P<0.0001$) in the combined discovery and replication cohorts.

Conclusions—We provide evidence that the *KCNQ1* rs2074238 polymorphism is an independent risk modifier with the minor T-allele conferring protection against cardiac events in patients with LQTS. This finding is a step toward a novel approach for risk stratification in patients with LQTS. **(*Circ Cardiovasc Genet.* 2013;6:354-361.)**

Key Words: association studies ■ genetics ■ ion channel ■ long-QT syndrome ■ polymorphism ■ risk factor

Long-QT syndrome (LQTS) is an uncommon hereditary cardiac disease characterized by delay in ventricular repolarization leading to a prolongation of the QT interval on ECG. The most frequent forms are autosomal-dominant. The majority of genotype-positive patients are carriers of private heterozygous mutations in the genes *KCNQ1* (LQT1, 40%–50%)

Received July 13, 2012; accepted June 21, 2013.

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The online-only Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.113.000023/-/DC1.

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and *KCNH2* (LQT2, 35%–45%) encoding the α-subunits of potassium channels.[1]

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Clinical Perspective on p 361

One of the most puzzling features of LQTS is the heterogeneity of clinical manifestations within family members who carry the same mutation.[1] The phenotypic differences have been explained so far in terms of variable penetrance,[2,3] but this is too vague an explanation. Accordingly, the presence of common genetic variants (polymorphisms) acting as modifiers and imprecisely but commonly referred to as modifier genes has been postulated.

Although many mutation carriers are symptomatic, with syncope, cardiac arrest (CA), or sudden cardiac death (SCD), others remain asymptomatic even without treatment. The interfamilial phenotypic variability can be associated with the nature of the mutations (allelic heterogeneity) and the disease-causing genes (locus heterogeneity). However, variable disease expression is observed even among patients carrying an identical mutation.[2,3] This intrafamilial variability, which cannot be explained by mutational heterogeneity, emphasizes the important role of modifying and triggering factors in the clinical phenotype.[4–8]

For patients with LQTS, risk of cardiac events (CEs) is significantly correlated with the extent of the QT interval prolongation.[9,10] Because of its dependence on heart rate, the QT interval needs to be corrected by heart rate (QTc). QTc is a complex quantitative trait with estimated genetic heritability of ≈30% to 50%.[11] Recent genome-wide association studies[12–14] and other investigations[15,16] have shown that genetic variants in different genes, including those coding for ionic channels (*KCNQ1*, *KCNH2*, *KCNE1*, *KCNJ2*, and *SCN5A*), or the neuronal nitric oxide synthase 1 regulator, CAPON (*NOS1AP*), can modulate QTc in healthy populations.

Individual sensitivity to sympathetic activation is another risk factor for CEs, and especially for LQT1 patients, most CEs occur under physical and emotional stress.[17] It has been suggested that functional polymorphisms in genes encoding adrenergic receptors (*ADRB1*, *ADRB2*, and *ADRA2C*) may contribute to an increasing arrhythmic risk in Finnish and South African (SA) LQT1 founder populations.[5,7,18]

It was on this background that we postulated that single nucleotide polymorphisms (SNPs) influencing either QTc duration or adrenergic responses might contribute to explain the striking clinical heterogeneity observed in LQTS, even within patients harboring the same mutation. Accordingly, we have assessed a cohort of patients from France, Italy, and Japan, with known heterozygous LQT1 or LQT2 mutations, to address whether polymorphisms act as genetic modifiers of clinical severity, by either increasing or reducing the risk of CE. As most of the previous studies were performed in patients of European descent, we also investigated whether the same polymorphisms were involved in European and Japanese patients with LQTS.

To validate the results obtained in this cohort, we selected the SNPs associated with arrhythmic risk or QTc duration for replication in 2 additional independent and well-described founder populations from South Africa and Finland.[19,20]

Methods

Study Population and Inclusion Criteria

Discovery Cohort

This study involved patients with clinically and molecularly diagnosed LQTS (LQT1 or LQT2) and represented a collaborative project comprising French, Italian, and Japanese referral centers. LQTS probands, mainly symptomatic patients, and their relatives were referred to our laboratories for genetic evaluation and enrolment in our study. The smallest pedigrees are nuclear families with first-degree relatives, which included the parent with LQTS and his or her offspring with LQTS. The largest kindreds are families with several generations. From the clinical data of families with LQTS, we selected duos composed of 2 relatives harboring the same LQT1 or LQT2 heterozygous mutation, one of whom experienced CEs, the other of whom was asymptomatic. One family with 2 heterozygous mutations was included in the study because both symptomatic and asymptomatic patients were carriers of both mutations.

Patients had undergone a clinical evaluation and cardiovascular examination, including a 12-lead ECG and 24-h Holter recording. LQTS was diagnosed on the basis of a Schwartz score ≥3.5[21] and a positive molecular screening. CEs were syncope (fainting spells with temporary but complete loss of consciousness), aborted CA (requiring resuscitation), and SCD. Symptomatic patients are considered those who experienced one or more CEs before 35 years of age. Patients with a first event after 35 years of age or with a drug-induced event were excluded from the study. Most LQTS mutation carriers, even when still asymptomatic, are currently treated with β-blockers; given their overall high efficacy in preventing CE,[22] it is impossible to know whether these individuals would have remained asymptomatic without therapy. Accordingly, we included in the study only asymptomatic mutation carriers >35 years of age and without therapy or treated after 35 years of age. Only families composed of patients symptomatic and asymptomatic according to these criteria were included. When several family members were available, we selected those most closely related, who would be expected to have fewer genetic differences. Moreover, when it was possible, we selected family members of the same sex. Thus, we selected 112 duos. Population characteristics (age, sex, ethnicity, ECG parameters, treatment, and history of CE) have been collected (Table 1). In total, there were 56 French, 15 Italian, and 41 Japanese duos. All patients and family members taking part in this study gave their informed consent for the genetic study, which was approved by the ethics committee.

Replication Populations

Two independent LQT1 founder populations were used to validate the findings obtained in the discovery cohort. Founder populations, characterized by a single ancestor affected by LQTS and a large number of individuals and families who are all related to the ancestor and thereby carry the same disease-causing mutation, represent a powerful human model for studying the role of modifier genes in LQTS.[23] As differences in clinical severity cannot be attributed to different underlying mutations, the most likely contributing factors are modifier genes. The SA *KCNQ1*-Ala341Val (SA-LQT1) founder population is characterized by unusual clinical severity, with 79% symptomatic mutation carriers and a mean age at first CEs of 6 years. Furthermore, 17% of the symptomatic subjects experienced CA/SCD.[19] The genotyped population was composed of 111 symptomatic and 41 asymptomatic patients with LQTS.

The Finnish *KCNQ1*-Gly589Asp (Finnish-LQT1) founder population represents the most common autosomal-dominant LQTS allele in Finland with 80 multigenerational families.[20] At variance with the SA-LQT1 population, only 30% of the mutation carriers were symptomatic for CEs.[20] There were 63 symptomatic and 121 asymptomatic genotyped patients with LQTS in this population.

These founder populations were previously described in detail,[19,20] and the definition of CEs, symptomatic or asymptomatic status, was essentially the same as that of the discovery cohort.

Table 1. Characteristics of all LQTS Mutation Carriers From the Discovery Cohort

	European	Japanese	All
LQT1	84 (59.2%)	36 (43.9%)	120 (53.6%)
LQT2	56 (39.4%)	46 (56.1%)	102 (45.5%)
LQT1+LQT2	2 (1.4%)	...	2 (0.9%)
Women	74 (52.1%)	43 (52.4%)	117 (52.2%)
Symptomatic	39 (52.7%)	26 (60.5%)	65 (55.6%)
Men	68 (47.9%)	39 (47.6%)	107 (47.8%)
Symptomatic	32 (47.1%)	15 (38.5%)	47 (43.9%)
Asymptomatic/symptomatic sex			
Women/women	18 (25.4%)	9 (22.0%)	27 (24.1%)
Men/men	15 (21.1%)	7 (17.1%)	22 (19.6%)
Women/men	17 (23.9%)	8 (19.5%)	25 (22.3%)
Men/women	21 (29.6%)	17 (41.5%)	38 (33.9%)
Age at first ECG, y			
Asymptomatic	49±12	48±10	48±11
Symptomatic	19±12	18±9	19±11
Cardiac events			
Syncope	58 (81.7%)	41 (100%)	99 (88.4%)
Cardiac arrest and sudden cardiac death	13 (18.3%)	...	13 (11.6%)
Age at the time of the first cardiac event, y			
All symptomatic patients	14±8	13±5	13±7
Women	17±9	13±5	15±7
Men	11±7	11±5	11±6
LQT1	12±8	11±5	12±7
LQT2	17±8	14±5	16±7
QTc, ms			
Women	480±39	502±50	483±44
Men	473±47	496±56	470±49

Data presented as n (%) or mean±SD. LQTS indicates long-QT syndrome.

Selection and Genotyping of Studied Polymorphisms

On the basis of recently reported association studies, we selected 25 polymorphisms described as influencing either QTc in healthy populations (21 SNPs) or adrenergic responses (2 SNPs and 2 deletions). We chose polymorphisms that gave the most robust and reproducible results and that were not in strong pairwise linkage disequilibrium (LD; $r^2<0.6$) in European populations, based on data available in the literature and databases (Table 2; Table I in the online-only Data Supplement). SNPs found to be associated with clinical status or QTc in the discovery cohort were then investigated in 2 additional independent replication populations.

Polymorphisms were genotyped in the discovery cohort either by using high-resolution melting with an unlabeled probe, Tm shift primers, fluorescence resonance energy transfer, or Taqman assays (Applied Biosystems) on a LightCycler 480 System (Roche), or by sequencing on a ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). All selected polymorphisms were also directly sequenced in a subset of 23 DNA samples. Genotyping by any of these methods and by direct sequencing yielded identical results. Six internal controls with known genotypes were systematically introduced into each 96-well plate. In the SA-LQT1 population, polymorphisms were genotyped by Taqman assay on 7900 HT Fast Real Time PCR System (Applied Biosystems), and in a subset of samples, direct sequencing was performed as well, with the same results. In the Finnish LQT1 population, genotyping was performed using the Sequenom iPLEX Gold assay (MALDI-TOF mass spectrometry, MassARRAY Analyzer Compact; Sequenom Inc). Primers and methods used for genotyping are listed in Tables II and III in the online-only Data Supplement.

Statistical Analysis

Deviation from the Hardy–Weinberg equilibrium was evaluated by an exact test separately in symptomatic and asymptomatic patients. We calculated pairwise LD statistics (r^2) using the Haploview program. Allele frequencies were estimated by gene counting separately in each population and subgroup (asymptomatic and symptomatic). We normalized QTc by normal quantile transformation and used this z-transformed variable for analysis.[24] As QTc is influenced by age[25] and sex,[26] we included these 2 covariates in the standard linear regression model to generate residuals. Next, we used these multivariate-adjusted residuals for the z-transformed variable to test for potential association with polymorphisms, clinical status (symptomatic versus asymptomatic), or severity of CEs (CA and SCD versus syncope). We also adjusted for ethnicity (Europeans or Japanese), and we used residuals (QTc adjusted for age and sex) as covariables to test for association between SNP and clinical status whenever appropriate. Comparison of age at the first CEs among symptomatic subgroups was performed by unpaired Student *t* test and log-rank test. Logistic regression was used to test the effect of QTc on severity of CEs among symptomatic patients. The association of polymorphisms with the binary clinical status, that is, asymptomatic versus symptomatic, or with the quantitative QTc trait was assessed by use of the generalized estimating equation (GEE) technique to account for correlation among related individuals.[27] In these GEE analyses, the so-called working independence matrix was used. For the binary trait analysis in the discovery cohort, the GEE results were compared with those obtained from a conditional logistic regression analysis that dealt with the matched case–control design of our family duos study. Both methods yielded similar results (data not shown). In the founder populations, GEE results obtained from the working independence matrix were compared with those obtained from a working correlation matrix derived from the kinship coefficients between individuals. Both analyses led to comparable results (data not shown). Only results derived from the working independence matrix were reported thereafter.

Because of the size of our cohort, association analyses were conducted under the dominant model. Odds ratios (ORs) with 95% confidence intervals were calculated. One-sided *P* values were reported under the hypotheses that polymorphisms that prolong QTc or increase response to adrenergic stress are associated with a higher risk of CEs, and polymorphisms that diminish QTc or decrease response to adrenergic stress are associated with a lower risk of CEs. *P* values were corrected for multiple testing using the Bonferroni procedure, and those lower than 2×10^{-3} (=0.05/25) were considered statistically significant in the discovery cohort. The GEE method was also used to perform analyses in the 2 founder populations. Results obtained in each population were combined in a meta-analysis. Based on the different design of the three populations studied, random-effect meta-analysis relying on the inverse-variance weighting was conducted. Homogeneity of associations across the discovery cohort and the 2 replication populations was evaluated using the Cochran's *Q* test method. All analyses were performed using R statistical software version 2.12.1 except where indicated.

Results

Study Discovery Population

A total of 60 LQT1 (53.6%) and 51 LQT2 (45.5%) duos, as well as 1 family with 2 heterozygous mutations (LQT1 and LQT2), were included (Table 1). There were 107 men (47.8%) and 117 women (52.2%). The mean QTc interval was longer in women (483±44 versus 470±49 ms; *P*=0.01). The mean QTc interval of European (French and Italian) and Japanese patients was almost identical (477±43 and 476±53 ms;

Table 2. Selected Polymorphisms

Gene	rs number	Location	Amino Acid Change	Tested Effect*
ADRA2C	rs61767072	Exon	p.Gly322_Pro325del	At risk
ADRB1	rs1801252	Exon	p.Ser49Gly	Protective
ADRB1	rs1801253	Exon	p.Arg389Gly	Protective
ADRB2	rs28365031	Exon	p.Glu307_Glu309dup	At risk
ATP1B1	rs10919071	Intron	...	Protective
KCNE1	rs1805128	Exon	p.Asp85Asn	At risk
KCNH2	rs1805123	Exon	p.Lys897Thr	Protective
KCNH2	rs3778873	Intron	...	Protective
KCNH2	rs3807375	Intron	...	At risk
KCNH2	rs3815459	Intron	...	At risk
KCNJ2	rs17779747	3′-Downstream	...	Protective
KCNQ1	rs12296050	Intron	...	At risk
KCNQ1	rs2074238	Intron	...	Protective
KCNQ1	rs757092	Intron	...	At risk
NOS1AP	rs10494366	Intron	...	At risk
NOS1AP	rs12029454	Intron	...	At risk
NOS1AP	rs12143842	5′-Upstream	...	At risk
NOS1AP	rs16857031	Intron	...	At risk
NOS1AP	rs4657139	5′-Upstream	...	At risk
NOS1AP	rs4657178	Intron	...	At risk
CNOT3	rs36643	5′-Near gene	...	Protective
PLN	rs11970286	5′-Upstream	...	At risk
PLN	rs12210810	5′-Upstream	...	Protective
SCN5A	rs12053903	Intron	...	Protective
SCN5A	rs1805124	Exon	p.His558Arg	At risk

*Minor alleles were considered at-risk alleles when they increased QTc and the risk of cardiac events, or enhanced adrenergic responses, whereas they were considered protective alleles when they decreased QTc and risk of cardiac events, or reduced adrenergic responses, according to previous studies. Minor alleles were defined according to allele frequencies in Europeans.

P=0.59). Symptomatic patients had a mean age at the first CE of 13±7 years, and 11.6% experienced CA and SCD (n=13), whereas the remaining 88.4% had only syncope (n=99).

The mean age at the first CE was lower in men than in women (11±6 versus 15±7 years; P<0.0007 for t test and P<0.0002 for log-rank test) and in LQT1 than in LQT2 patients (12±7 versus 16±7 years; P<0.003 for t test and P=0.01 for log-rank test) as illustrated by the Kaplan–Meier curves (Figure I in the online-only Data Supplement).

Association Between QTc and Clinical Status in the Discovery Population

As expected, QTc was longer in symptomatic than in asymptomatic LQTS mutation carriers (491±49 versus 462±40 ms; P<0.007). Patients who experienced CA or SCD were prone to have a QTc longer than in other symptomatic mutation carriers (501±58 versus 489±48 ms), but the association failed to reach statistical significance (P=0.12).

Polymorphism Studies in the Discovery Population

We observed a weak deviation from Hardy–Weinberg equilibrium for rs10494366 in European patients (P=0.02 for asymptomatic and P=0.04 for symptomatic patients) and for four polymorphisms in the Japanese asymptomatic group (P=0.02 for rs1805124; P=0.03 for rs17779747 and rs10919071; P=0.04 for rs3778873). However, after Bonferroni correction for multiple testing, these deviations were no longer statistically significant.

Polymorphism Frequencies and LD

Allele frequencies for all studied polymorphisms in European and Japanese patients with LQTS are shown in Table IV in the online-only Data Supplement. As reported in databases, allele frequencies of some polymorphisms were quite different between ethnic groups (Tables I and IV in the online-only Data Supplement). As expected from our SNP selection, no strong pairwise LD between polymorphisms was observed in European or Japanese populations (Figure II in the online-only Data Supplement).

Association Between Polymorphisms and Clinical Status

Allele frequency distributions were similar between the symptomatic and asymptomatic groups except for the *KCNQ1* rs2074238 polymorphism (Table IV in the online-only Data Supplement). Indeed, the *KCNQ1* rs2074238-T allele was significantly less frequent in symptomatic than in asymptomatic LQTS patients (0.04 versus 0.10 in Europeans; P<0.002) and associated with a protective OR of 0.38 (0.19–0.73; Table 3 and Tables IV and V in the online-only Data Supplement). This effect, present in the European patients, was not observed in the Japanese cohort as this SNP was not polymorphic.

Association Between Polymorphisms and QTc

None of the studied SNPs demonstrated significant (at the Bonferroni-corrected threshold) association with QTc duration (data not shown). Nevertheless, 2 SNPs, rs12029454 and rs2074238, showed suggestive evidence of association with P<0.05. The strongest association (P=0.006) with QTc duration was observed for the *NOS1AP* rs12029454, where carriers of the A-allele had a longer QTc than noncarriers (484±50 versus 471±44 ms; Table 4 and Table VI in the online-only Data Supplement). Additionally, the *KCNQ1* rs2074238 T-allele tended to be associated with a shorter QTc than non-T-carriers (464±35 versus 479±44 ms; P=0.04; Table 4). Of note, after adjustment for QTc, the effect of rs2074238 on the risk of CEs was barely modified (OR, 0.36 [0.17–0.78]; P=0.005). These 2 SNPs were then passed to the replication study.

Replication Study

We further investigated the associations observed for *KCNQ1* rs2074238 with CEs and QTc and that of *NOS1AP* rs12029454 with QTc in 2 additional independent founder populations, 1 from South Africa and the other from Finland.

In the SA-LQT1 founder population, the *KCNQ1* rs2074238 T-allele was less frequent in symptomatic than in asymptomatic individuals (0.01 versus 0.06) and associated with a protective OR of 0.20 (0.09–0.44), P<0.0001 (Table 3). This association was consistent with that observed in the discovery samples. The same trend of association was observed in the

Table 3. Association Between SNP and Clinical Status in LQTS Mutation Carriers

Gene	SNP	Population	Genotypic Status	Asymptomatic	Symptomatic	*P* Value§	OR (95% CI)
KCNQ1	rs2074238	Discovery*	CC	98 (87.5%)	106 (94.6%)	1.98×10^{-3}	0.38 (0.19–0.73)
			CT	14 (12.5%)	6 (5.4%)		
		South African	CC	36 (87.8%)	108 (97.3%)	3.13×10^{-5}	0.20 (0.09–0.44)
			CT	5 (12.2%)	3 (2.7%)		
		Finnish	CC	109 (90.1%)	58 (92.1%)	0.33	0.78 (0.26–2.34)
			CT	12 (9.9%)	5 (7.9%)		
		Meta-analysis (replication†)‡	...	...	...	0.07	0.37 (0.10–1.42)
		Meta-analysis (discovery and replication†)	...	...	...	1.68×10^{-3}	0.36 (0.18–0.71)

CC represents the major homozygous genotype; CI, confidence interval; CT, the heterozygous genotype; LQTS, long-QT syndrome; and SNP, single-nucleotide polymorphism.

*Pooled population (European and Japanese).

†South African and Finnish.

‡Heterogeneity: I^2=0.75, P=0.05.

§Not corrected for multiple tests.

Finnish population (OR, 0.78 [0.26–2.34]), although it did not reach statistical significance (Table 3). The meta-analysis of the 3 studies suggested moderate[28] (I^2=0.51) but not significant (P=0.13) heterogeneity in the *KCNQ1* rs2074238 association with CE risk. The resulting overall association was significant (OR, 0.36 [0.18–0.71]; P<0.002).

The association of rs2074238 with QTc was replicated in the 2 additional populations. In both SA and Finnish, carriers of the rs2074238 T-allele demonstrated shorter QTc than noncarriers (467±23 versus 488±43 ms, P=0.008; 445±31 versus 465±34 ms, P=0.003, respectively) (Table 4). The meta-analysis of these 2 populations showed significant association (P<0.0002). Furthermore, performing meta-analysis of these 2 populations together with the discovery samples demonstrated an overall statistical evidence of P<0.0001 for the association of rs2074238 with QTc, without evidence for heterogeneity (I^2=0; P=0.78).

After adjustment for QTc, the combined OR for CEs associated with the rs2074238 T-allele derived from the meta-analysis of the 3 studies was 0.34 (0.19–0.61; P<0.0002).

Table 4. QTc Duration in LQTS Mutation Carriers According to Replicated SNPs

			AA		Aa and aa		
Gene	SNP	Population	QTc, ms	n	QTc, ms	n	*P* Value**
KCNQ1	rs2074238	Discovery*	479±44	120	464±35	18	0.04
		South African	488±43	101	467±23	6	8.14×10^{-3}
		Finnish	465±34	152	445±31	15	2.95×10^{-3}
		Meta-analysis (replication†)‡	474±40	253	451±30	21	1.37×10^{-4}
		Meta-analysis (discovery and replication†)	476±41	373	457±33	39	3.43×10^{-5}
NOS1AP	rs12029454	Discovery§	471±44	125	484±50	95	6.08×10^{-3}
		South African	483±37	88	501±63	20	0.18
		Finnish	459±32	97	468±37	70	0.08
		Meta-analysis (replication†)‖	470±36	185	475±46	90	0.05
		Meta-analysis (discovery and replication†)	471±40	310	483±42	185	1.46×10^{-3}

Data presented as mean±SD. AA represents the major homozygous genotype (corresponding to CC for rs2074238 and GG for rs12029454); Aa and aa, the minor allele (T for rs2074238 and A for rs12029454) in the heterozygous or homozygous state. LQTS indicates long-QT syndrome; and SNP, single-nucleotide polymorphism.

*European population (rs2074238 is not polymorphic in the Japanese population).

†South African and Finnish.

‡Heterogeneity: I^2=0, P=0.74.

§Pooled population (European and Japanese).

‖Heterogeneity: I^2=0, P=0.84.

**Not corrected for multiple tests.

In both SA-LQT1 and Finnish replication samples, carriers of the minor *NOS1AP* rs12029454 A-allele tended to have longer QTc compared with noncarriers (501±63 versus 483±37 and 468±37 versus 459±32, respectively), consistent with that observed in our discovery cohort, and the meta-analysis of the 2 replication populations led to a P value of 0.05 (Table 4). When the meta-analysis was performed on the 3 populations, there was no evidence for heterogeneity (I^2=0; P=0.87), although there was an association of rs12029454 with QTc (P<0.002).

Discussion

The present study provides strong evidence that the *KCNQ1* rs2074238 T-allele is associated with a decreased risk for CEs among patients affected by LQTS. This SNP had been previously identified as modulating QTc duration in healthy individuals, Europeans or of European descent,[13,29,30] which is also the case in our LQTS population.

The correct identification, within LQTS family members carrying the same mutation, of those at higher or lower risk for life-threatening CEs is still a daunting problem for the physicians involved. The rs2074238 SNP could contribute to explain some of the puzzling phenotypic variability among patients with LQTS and might improve the cardiac risk prediction already provided by QTc, sex, age, or type of disease-causing mutation.[9,10,31–33] This finding may thus have an impact on clinical management and, together with the SNPs recently reported to increase arrhythmic risk,[5–8,34,35] represents a significant step marking the progressive transition from gene-specific,[9] to mutation-specific,[31,32] and now toward SNP-specific risk stratification, a truly exciting evolution of genotype–phenotype correlation studies.

Genetic Findings

The association between rs2074238 and CEs, which was restricted to European patients in the discovery cohort, remained significant after correction for multiple testing and was replicated in the SA-LQT1 founder population, as well as in the meta-analysis. The same trend of association was observed in the Finnish-LQT1 population but failed to reach the 0.05 significance threshold. The lack of replication in this population could have been predicted because of the expected ceiling effect, that is, if the disease-causing mutation is associated with few events, it becomes difficult to demonstrate a statistically significant protective effect unless thousands of patients are studied. Because this SNP was not polymorphic in the Japanese patients with LQTS, the association with CEs in a non-European population could not be tested. Because of the low prevalence of this SNP among our patients with LQT2, it seems prudent to say that this protective effect is primarily related to the patients with LQT1. Because the rs2074238 T-allele has a frequency of 6.5% in Europeans, it is predicted to influence phenotypic differences in <10% of patients with LQTS.

In addition to its clear protective effect (ie, lower arrhythmic risk), we observed that the minor *KCNQ1* rs2074238 T-allele was associated with shorter QTc in the 3 investigated cohorts. We also showed that its protective effect on CE risk was not only because of its impact on QTc duration.

There are insufficient data available to postulate a mechanism underlying our findings. One reasonable consideration stems from the fact that *KCNQ1* rs2074238 (c.386+18089C>T) is located in intron 1, which shows only modest LD (r^2<0.50) with known *KCNQ1* SNPs[36]; the Encyclopedia of DNA Elements project[37] and the RegulomeDB variant classification[38] provide strong support for a functional role for rs2074238 in regulating *KCNQ1* expression. Further experimental data, including quantitative expression analyses, are needed to test this possibility and to clarify the underlying mechanism(s). After confirmation of a functional role for rs2074238 in regulating *KCNQ1* expression, it would be of interest to assess whether this effect could depend on whether the rs2074238 T-allele is on the same haplotype as the LQT1-mutated allele (cis-effect) or associated with the nonmutated allele (trans-effect), as recently reported for SNPs located in *KCNQ1* 3′-untranslated region.[35]

We found another SNP, *NOS1AP* rs12029454, known for influencing QTc in the general population, which showed suggestive evidence of association with longer QTc duration in our LQTS discovery and validation cohorts. The importance of NOS1AP in modulating the QTc is well recognized in healthy subjects,[12–14,29,30] as well as in LQTS patients.[8,34]

Study Design

To eliminate the otherwise unavoidable bias introduced by studying different LQTS mutations with varying severity, we used 2 different approaches: (1) the use of duos sharing the same disease-causing mutations; (2) the use of founder populations. The second approach has already been validated in previous studies. Indeed, in the SA-LQT1 population carrying the *KCNQ1*-A341V founder mutation, we demonstrated that some common polymorphisms in *NOS1AP* increased the clinical severity and QTc duration.[8] Similarly, rs1805128 in *KCNE1* has been associated with QTc prolongation in 712 LQT1 or LQT2 Finnish founder mutation carriers derived from 126 LQTS families.[39] These results were observed in ethnically isolated founder populations from South Africa and Finland, an unusual situation given that most LQT1 or LQT2 patients carry private mutations. Studies on mixed populations are, therefore, also important to determine whether the modifying effect of certain SNPs could apply to different LQTS mutations, and not be restricted to a specific one. Such a study on 3 *NOS1AP* SNPs was conducted in 901 LQTS patients of European descent from 520 families,[34] and essentially confirmed the results reported in the SA-LQTS founder population.[8] Thus, findings in founder populations, which are particularly suitable to identify modifier genes, can be translated to the general LQTS population with a reasonable degree of confidence.

This is the first study to test the effect of a large number of polymorphisms on phenotypic variability and QTc interval duration in LQTS patients selected for representing both asymptomatic and symptomatic carriers of the same mutation within each family, thus constituting a matched case–control study. In this way, we avoided the confounding effects of genetic and allelic heterogeneity that is present whenever a study involves variable numbers of patients with multiple disease-causing mutations, each associated with different cardiac risk.

Comparison With Published Data

Of the several polymorphisms previously shown to affect QTc duration in large healthy populations,[12–16,29,30] only 2 had a similar effect in our LQTS cohorts. This should not be surprising because, although a modest prolonging effect could be easily demonstrable in individuals with a normal QTc, such a small change is likely to be overshadowed by the major effect of the underlying disease-causing mutation. In other words, a 5- to 10-ms prolongation, which may be noticed in populations with a normal QT interval, and a modest standard deviation (usually ≈20 ms) would be totally lost in populations, like ours, with much longer mean QTc and a much greater SD (≈45 ms). Consequently, when powers were calculated using QUANTO software (http://hydra.usc.edu/gxe) to reproduce the effect of the various SNPs on QT prolongation, they ranged from 7% to 21% in the discovery cohort (Table VII in the online-only Data Supplement).

The SNPs within the adrenergic receptor genes *ADRB1* (rs1801252 and rs1801253),[5,7] *ADRA2C* (rs61767072),[5] and rs1805128 on *KCNE1*,[39] previously associated with arrhythmic risk in LQTS patients, were not validated in the present cohort. However, the rare T-allele of *KCNE1* rs1805128 tended to be more frequent in European symptomatic patients (0.05 versus 0.01; OR, 7.66 [1.21–48.31]; P=0.02) as previously observed (Table V in the online-only Data Supplement). Among *NOS1AP* SNPs, only rs12029454 was associated with QT prolongation, although no association was identified with CEs in the discovery cohort. Thus, we did not replicate the previously reported association with the occurrence of CEs for rs4657139,[8,34] rs16847548[8] (in strong LD with rs12143842 in Europeans; r^2>0.8), or rs10494366.[34] This lack of statistical replication could be because of the size of the population, the rarity of some variants, and thus insufficient power (Table VII in the online-only Data Supplement), but also to population specificities, and differences in genetic or environmental background. Differences in the clinical phenotypes could also contribute. For instance, the findings on the adrenergic receptor genes were obtained in patients selected on the basis of QTc changes during an exercise stress test,[7] and on the distribution of baroreflex sensitivity,[5] although for the *KCNE1* polymorphism, the association identified was limited to male patients.[39] It is likely that the effects of several SNPs on patient QT interval or cardiac risk are gene-, mutation-, or allele-dependent.[9,31,32,35] In addition, the risk is dependent on the ratio of SNP alleles with opposing effects, for example, reducing or favoring QT prolongation, and more complex stratifications should be performed in the future when those SNPs with the greatest effects will be progressively validated.

Conclusions

The present demonstration of the existence of a common genetic variant capable of decreasing the risk for life-threatening arrhythmias among patients affected by the LQTS represents a significant step in the never-ending quest for a more thorough understanding of the complex relationship between genotype and phenotype.

Much progress has been made during the last 10 to 15 years, and now our new findings suggest that we are moving toward a phase in which risk stratification will be further refined by identifying a cluster of polymorphisms capable of modifying the mutation-specific risk in either direction. This phase will require the identification of several other polymorphisms modifying clinical outcome. The impact on clinical management will not be immediate, but there is little doubt that a significant refinement in the strategy for risk stratification for LQTS is well under way.

Acknowledgments

We are grateful to Dr Jean-Sylvain Hermida, Dr Joël Kamblock, Dr Jean-Marc Lupoglazoff, Dr Philippe Maury, Dr Dagmar I. Keller, Dr Elizabeth Villain, and Dr Philippe Charron, who provided clinical data on patients with LQTS. The authors also thank Pinuccia De Tomasi, BS, for expert editorial support.

Sources of Funding

This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Université Pierre and Marie Curie, a Région Ile-de-France fellowship to Dr Duchatelet; a European Commission Grant 221685 to Drs Guicheney and Peat; funding from the Fédération Française de Cardiologie and the Société Française de Cardiologie for Drs Denjoy and Guicheney; a Telethon-Italy grant GGP07016 and National Institutes of Health grant HL068880 to Drs George, Schwartz, and Crotti; the Finnish Foundation for Cardiovascular Research to Dr Swan; the Finnish Cultural Foundation to Dr Lahtinen; and the Sigrid Juselius Foundation to Dr Kontula.

Disclosures

None.

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CLINICAL PERSPECTIVE

The striking heterogeneity of the clinical manifestations present even among family members carrying the same mutation is 1 of the most puzzling features of long-QT syndrome. We and others have postulated a major role for common genetic variants acting as modifiers. Here, we performed a case–control study in 112 patient duos with long-QT syndrome by genotyping 25 polymorphisms associated mostly with QTc duration and then validated the findings in 2 independent founder populations. We identified a *KCNQ1* single-nucleotide polymorphism significantly associated with a decreased risk for cardiac events in the combined discovery and replication cohorts. The identification of a protective modifier represents an important step toward single-nucleotide polymorphism–specific risk stratification. On the one hand, it contributes to a better understanding of the complex relationship between genotype and phenotype; on the other, it represents 1 more step toward the necessary identification of a cluster of polymorphisms capable of modifying risk in either direction.

Journal of the American College of Cardiology
© 2013 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 62, No. 17, 2013
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2013.05.081

Significance of Non-Type 1 Anterior Early Repolarization in Patients With Inferolateral Early Repolarization Syndrome

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Objectives The aim of this study was to investigate the significance of non-type 1 anterior early repolarization (NT1-AER) combined with inferolateral early repolarization syndrome (ERS).

Background Inferolateral ERS might be a heterogeneous entity, although it excludes type 1 Brugada syndrome (BS).

Methods Of 84 patients with spontaneous ventricular fibrillation, 31 ERS patients were divided into 2 groups. The ERS(A)-group consisted of inferolateral ER and NT1-AER—that is, notching or slurring with J-wave ≥1 mm at the end of QRS to early ST segment in any of V_1 to V_3 leads, in which the ST-T segment did not change to a coved pattern in the standard and high costal (second and third) electrocardiographic recordings even after drug provocation tests (n = 12). The other, ERS(B)-group, showed only inferolateral ER (n = 19). Clinical characteristics and outcomes were compared between the ERS groups, 40 patients with type-1 BS (BS-group), and 13 patients with idiopathic ventricular fibrillation lacking J-wave (IVF-group).

Results Ventricular fibrillation occurred during sleep or near sleep in 10 of 12 patients in ERS(A)-group and in 22 of 40 patients in BS-group but in 2 of 19 patients in ERS(B)-group and in 1 of 13 patients in IVF-group (ERS[A] vs. ERS[B], p < 0.0001). Ventricular fibrillation recurrence was significantly higher in ERS(A)-group (58%), particularly in patients with J waves in the high lateral lead, and BS-group (55%), compared with ERS(B)-group (11%) and IVF-group (15%) (ERS[A] vs. ERS[B], p = 0.012).

Conclusions Inferolateral ERS comprises heterogeneous ER subtypes with and without NT1-AER. Coexistence of NT1-AER was a key predictor of poor outcome in patients with ERS. (J Am Coll Cardiol 2013;62:1610–8) © 2013 by the American College of Cardiology Foundation

J-wave or early repolarization (ER) is a common electrocardiographic finding that affects 1% to 13% of individuals and is characterized by the elevation of the J point with notching or slurring on electrocardiogram (ECG) followed by ST-segment elevation (1–3). Although this condition had been considered benign, Haïssaguerre et al. (1) demonstrated patients with J waves in the inferolateral lead (I, II, III, aVL, aVF, and V_4 to V_6) were likely to be associated with idiopathic ventricular fibrillation (IVF) and reported this entity as inferolateral early repolarization syndrome (ERS) in 2008. Since then, the J-wave has come to be known as a possible indicator of an increased risk for death due to cardiac arrhythmia.

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Brugada syndrome (BS) is another clinical entity that causes sudden death because of ventricular fibrillation (VF) in patients with apparently structurally normal hearts and is characterized by coved ST-segment elevation in the right precordial leads (V_1 to V_3) (4,5). Yan and Antzelevitch, with a canine Brugada model (6), demonstrated that this peculiar ST-segment elevation in the anterior lead can be an expression of ER or J-wave caused by transient outward current (Ito)-mediated transmural differences in early phases

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Manuscript received April 18, 2013; accepted May 7, 2013.

of the action potential. Haïssaguerre et al. (1) defined inferolateral ERS as a disease characterized by J waves only in inferolateral lead and excluded patients with type 1 (coved) ST-segment elevation in leads V_1 to V_3 from this syndrome. However, patients with non-type 1 ST-segment elevation or J waves showing notching or slurring in the anterior leads can be theoretically included in this syndrome. Therefore, we investigated the significance of the non-type 1 anterior ER (NT1-AER) excluding coved ST-segment elevation in inferolateral ERS by comparing the clinical profile and long-term prognosis in patients with and without NTI-AER.

Abbreviations and Acronyms

BS = Brugada syndrome
coved-AER = coved anterior early repolarization
ECG = electrocardiogram
ER = early repolarization
ERS = early repolarization syndrome
ERS(A) = inferolateral early repolarization combined with non-type 1 anterior early repolarization
ERS(B) = pure inferolateral early repolarization without anterior early repolarization
ICD = implantable cardioverter-defibrillator
IVF = idiopathic ventricular fibrillation
LV = left ventricle/ventricular
NT1-AER = non-type 1 anterior early repolarization
RV = right ventricle/ventricular
VF = ventricular fibrillation

Methods

Study population. The study population consisted of 84 consecutive patients who were admitted to our hospital between 1992 and 2011 due to spontaneous idiopathic VF. Patients exhibited 3 distinct ECG patterns as follows: 31 had inferolateral ERS (ERS-group, 27 men, mean age: 42.0 ± 14.1 years); 40 had type 1 BS (BS-group, 40 men, mean age: 43.0 ± 12.2 years); and 13 had idiopathic VF without J waves (IVF-group, 8 men, mean age: 46.1 ± 12.7 years). None of the patients had structural heart disease, including arrhythmogenic right ventricular (RV) cardiomyopathy, which was confirmed by noninvasive studies (physical examination, 12-lead ECG, 87-lead body surface ECG, exercise stress test, signal-averaged ECG, echocardiography, and cardiac magnetic resonance imaging or computed tomography) and invasive studies consisting of coronary angiography including ergonovine/acetylcholine injection and right or left ventricular (LV) cineangiography. Patients with coronary artery spasm, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia, commotio cordis, drug-induced VF, and hypothermia were excluded in all groups. Abnormal QTc interval was defined as ≥460 ms and <340 ms during sinus rhythm.

Classification of inferolateral ERS, BS, and IVF groups. We divided the 31 patients with inferolateral ERS and a prior episode of VF into 2 groups according to their baseline ECGs and the results of the drug provocation tests with a sodium channel blocker. One group consisted of patients with an inferolateral ER combined with NT1-AER (ERS[A]-group) (i.e., anterior ER consisting of notching or saddleback ST-segment elevation in any of the right precordial leads). The other group consisted of a pure inferolateral ER without anterior ER (ERS[B]-group).

The presence of inferolateral ER, which was defined as an elevation of the J point in at least 2 leads, was evaluated by baseline 12-lead ECGs (25 mm/s and 10 mm/mV). The amplitude of the inferolateral J-wave or J-point elevation had to be at least 1 mm or 0.1 mV above the baseline level, either as QRS slurring or notching in any of the inferior (II, III, and aVF), lateral (V_4, V_5, and V_6), and high lateral (I and aVL) leads (1) in at least 1 ECG recording.

The NT1-AER was defined as upward/downward notching or downward slurring with an amplitude ≥1 mm at the end of QRS to early ST segment in any of the anterior leads (V_1, V_2, and V_3) in the baseline standard or high costal (second and third) ECG recordings or in those ECGs after drug provocation tests, which includes type-2 and type-3 Brugada-pattern ECG (4). The upward/downward notch in the anterior leads should have appeared between the late QRS and early ST period in the same timeframe as J waves in other leads in the same 12-lead ECG.

Coved AER was defined as type 1 Brugada ECG (4) or coved ST-segment elevation ≥2 mm followed by a positive or flat T-wave in any of anterior leads, either occurring spontaneously or after challenge with a sodium channel blocker. Patients with coved-AER were excluded from the ERS-group and included in the BS-group, although patients in the BS-group should have shown type 1 ECG with a negative T-wave spontaneously or after drug test according to consensus reports (4,5). The ERS-group excluded patients with bundle branch block of QRS duration ≥110 ms on the standard ECG. The IVF-group also excluded patients with J waves or ER in any leads (inferolateral ER, NT1-AER, and coved–AER) in the standard and high costal recordings even after drug provocation test.

Clinical profiles, electrocardiographic characteristics, and VF recurrences during follow-up were compared among the patients in the ERS(A), ERS(B), BS, and IVF groups.

Clinical data, ECG, genetic, and electrophysiological testing. Clinical data including age at the first episode of VF, sex, family history of sudden cardiac death at <45 years of age, activity of patients at VF, the location of leads showing ER, and prognosis were collected for all patients. We defined the state of patients at VF as "sleep" when VF occurred in a state of sleeping and as "near sleep" when VF occurred in a resting state without physical activity just after waking. During follow-up, patients were considered to have an arrhythmic event if VF was documented by implantable cardioverter-defibrillator (ICD) interrogation. An electrical storm was defined as ≥3 VF episodes within 24 h. The beginning of the follow-up period was at the time of the first VF event. In patients with recurrent arrhythmias, the choice of antiarrhythmic drugs was decided by the physician of the patient.

All ECGs were analyzed by 2 independent cardiologists (T.K., S.K.), and consensus was reached about the diagnosis. The J waves in the extensive lead were considered to be J waves in the inferior and lateral or high lateral leads.

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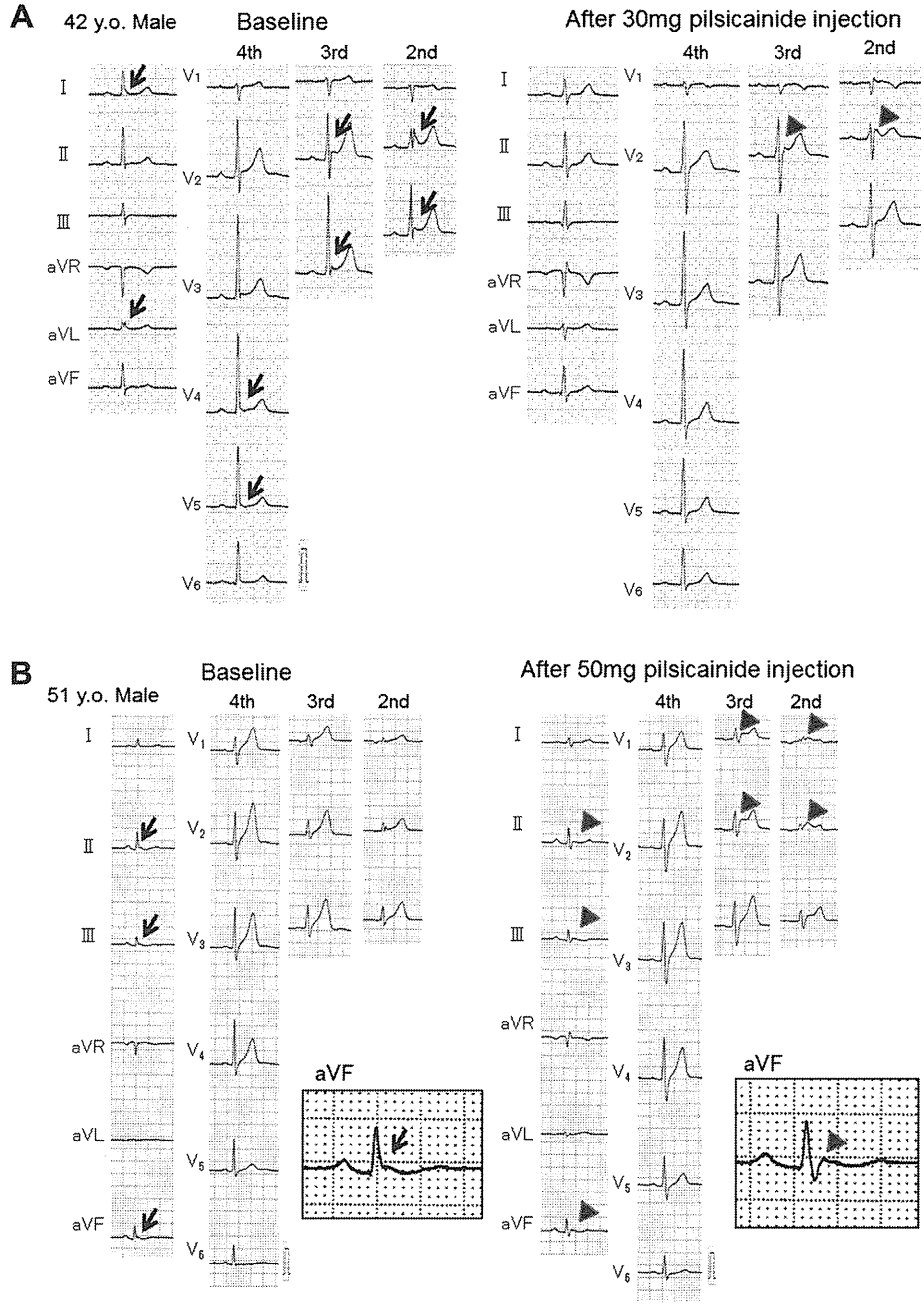

Figure 1 ECGs in the ERS(A)-Group

(A) At baseline, a 42-year-old man exhibited J waves **(arrows)** followed by ascending ST-segments in leads I, aVL, V_4, and V_5 in the standard (4th) recording and in leads V_2 and V_3 in the third (3rd) and second (2nd) intercostal recordings. After pilsicainide injection, all J waves in limb lead disappeared with appearance of S waves and an R-wave in lead aVR. Saddleback ST-segment elevation with slightly augmented J waves **(broad arrows)** were also noted in V_2 in the high intercostal spaces. He experienced an electrical storm 4 years after implantable cardioverter-defibrillator (ICD) insertion. **(B)** At baseline, electrocardiograms (ECGs) of a 51-year-old man exhibited J waves followed by horizontal or descending ST segments in leads II, III, and aVF **(arrows)**. There was no sign of coved or saddleback ST-segment elevation in any chest leads. After injection of 50-mg pilsicainide, saddleback ST-segment elevation **(broad arrows)** appeared in leads V_1 and V_2 in the second and third intercostal spaces with augmented J waves **(broad arrows)** preceded by newly appearing S waves in leads II, III, and aVF **(shown in expanded ECGs)**. He showed spontaneous VF 1 month after ICD insertion. **(C)** A 31-year-old man had J waves **(arrows)** followed by ascending ST-segments in leads I, II, III, aVF, V_4, V_5, and V_6 in the baseline standard (4th) ECG. Furthermore, upward/downward spiky notches $\geq$1 mm were identified at the end of QRS to early ST-segment in leads V_1 and V_2 only in the high costal (3rd and 2nd) recordings. After pilsicainide injection, J waves in leads I, aVL, and V_6 were augmented **(broad arrows)**, although other J waves were unchanged or slightly attenuated. He experienced an electrical storm 1 month after ICD insertion.

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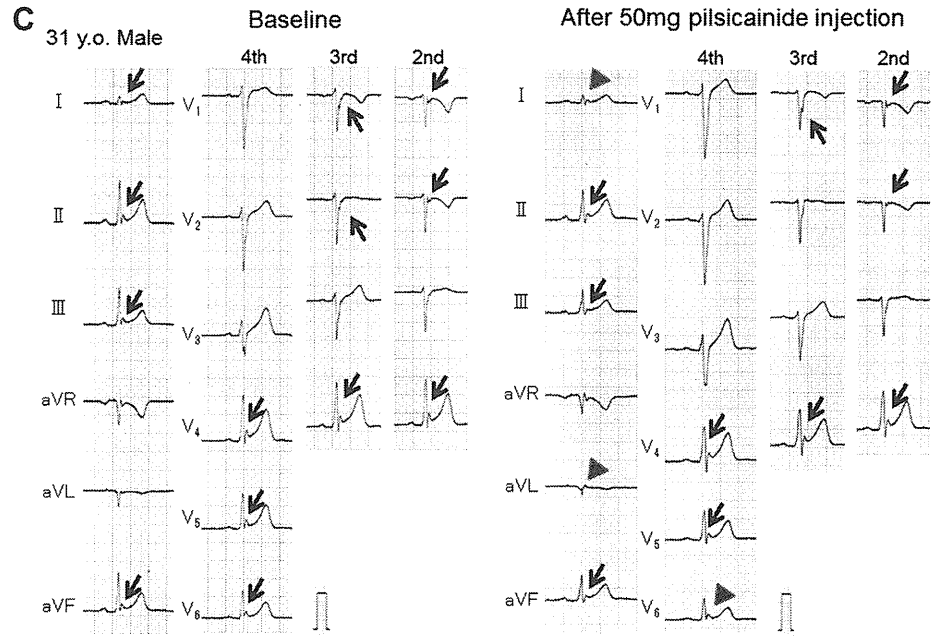

Figure 1 **Continued**

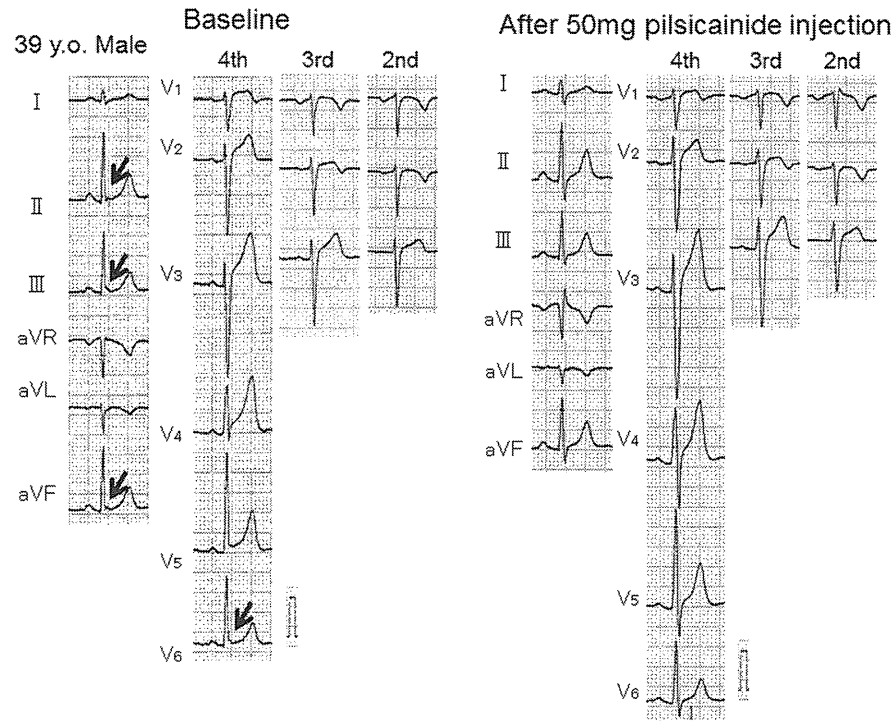

Figure 2 **ECGs in the ERS(B)-Group**

In the baseline electrocardiograms (ECGs) of this 39-year-old man, J waves were present in leads II, III, aVF, and V_6 **(arrows)**. After pilsicainide injection, they all disappeared with appearance of S waves. The ECGs in leads V_1 to V_3 during standard and high costal recording remained normal, even after pilsicainide injection. This patient experienced no ventricular fibrillation recurrence.

Electrophysiological study was conducted in 59 (ERS [A]: 6; ERS[B]: 10; BS: 32; and IVF: 11) patients, as previously reported (7). Patients with inducible VF were classified as inducible. Genetic testing for mutations in *SCN5A* gene was also performed in 43 (ERS[A]: 7; ERS [B]: 12; and BS: 24) patients, as previously described (8).

Drug provocation test. Drug provocation tests were conducted with pilsicainide (up to 1 mg/kg body weight at a rate of 5 to 10 mg/min), disopyramide (1.5 mg/kg, 10 mg/min), or flecainide (2 mg/kg, 10 mg/min) in all patients of the ERS and IVF groups during standard and high costal (second and third) ECG recordings. This study was approved by the institutional research board of National Cerebral and Cardiovascular Center.

Statistical analysis. Data were analyzed with JMP7 software (SAS Institute, Inc., Cary, North Carolina). Numeric values are presented as mean ± SD. The chi-square test, Student *t* test, or 1-way analysis of variance was performed as appropriate to test for statistically significant differences. Survival curves were constructed by the Kaplan-Meier method and compared by the log-rank test. A probability value of $p < 0.05$ was considered statistically significant.

Results

ECG findings of ERS(A) and ERS(B) groups. Figures 1 and 2 show typical ECGs at baseline and after drug provocation test, respectively, in patients with inferolateral ERS. Twelve of 31 (39%) patients in the inferolateral ERS group had NT1-AER in the right precordial leads in standard or high costal ECG recordings, spontaneously or after drug provocation tests: 7 (type 2: 4; type 3: 2; notch: 1) on the baseline standard ECGs; 4 (type 2: 2; type 3: 1; notch: 1) only on the high intercostal ECG recordings before drug provocation (Figs. 1A and 1C); and 1 (type 2: 1) on the high intercostal ECG after pilsicainide injection (Fig. 1B). None of these ECGs of patients including high costal recordings ever revealed the coved-AER pattern during follow-up. By contrast, 19 patients in the ERS(B)-group showed only inferolateral ER pattern spontaneously or after drug provocation test and in follow-up (Fig. 2).

Clinical profiles in each group. Clinical characteristics of the patients in each group are shown in Table 1. Most patients were male. Ventricular fibrillation developed during sleep or near sleep in 10 of 12 (83%) patients in ERS(A) (sleep: 8; near sleep: 2) and in 22 of 40 (55%) patients in the BS-group (sleep: 17; near sleep: 5), in contrast to only 2 of 19 (11%) patients (sleep: 2) in the ERS(B)-group (ERS[A] vs. ERS[B], $p < 0.0001$; and BS vs. ERS[B] $p = 0.0015$) and 1 of 13 (8%) patients (sleep: 1) in the IVF-group. Most patients in the ERS(B) and IVF-group had VF during activity.

The J waves were spontaneously noted in the high lateral, lateral, inferior, and extensive leads in 10, 7 ,6, and 5 patients, respectively, in the ERS(A)-group and in 3, 15, 15, and 12 patients, respectively, in the ERS(B)-group. After sodium channel blocker provocation, J waves disappeared or

Table 1 Patient Characteristics of the ERS(A), ERS(B), BS, and IVF Groups

	ERS (n = 31)				
Group	ERS(A) (n = 12)	ERS(B) (n = 19)	BS (n = 40)	IVF (n = 13)	p Value ERS (A) vs. ERS (B)
Clinical characteristics					
Age (yrs)	41.2 ± 11.4	42.5 ± 15.8	43.0 ± 12.2	46.1 ± 12.7	0.60
Men	9 (75%)	18 (95%)	40 (100%)	8 (62%)	0.27
FH of SCD	1 (8%)	0 (0%)	8 (20%)	2 (15%)	0.39
Sleep at the time of SCA (sleep, near sleep)	10 (8,2) (83%)	2 (2,0) (11%)	22 (17,5) (55%)	1 (1,0) (8%)	<0.0001
Electrocardiographic findings					
Location of J waves					
High lateral (I, aVL)	10 (83%)	3 (16%)	5 (13%)		0.0005
Lateral (V_4–V_6)	7 (58%)	15 (79%)	4 (10%)		0.25
Inferior (II, III, aVF)	6 (50%)	15 (79%)	8 (20%)		0.13
Extensive (inf+lat/high lat)	5 (42%)	12 (63%)	2 (5%)		0.29
Inferolateral J-wave with ascending ST-segment	3 (25%)	11 (58%)			0.14
Inferolateral J-wave with horizontal/descending ST-segment	9 (75%)	8 (42%)			0.14
J-wave elevation by sodium channel blocker	9 (75%)	0 (0%)			<0.0001
VF induction by EPS	3/6 (50%)	3/10 (30%)	26/32 (81%)	3/11 (27%)	0.61
Clinical outcome					
Follow-up (months)	90 ± 57	76 ± 46	104 ± 63	82 ± 50	0.76
VF recurrence	7 (58%)	2 (11%)	22 (55%)	2 (15%)	0.012
VF storm	5 (42%)	0 (0%)	10 (25%)	0 (0%)	0.0047
ICD insertion	12 (100%)	18 (95%)	38 (95%)	10 (77%)	1.0

Values are mean ± SD or n (%).

Ant = anterior lead; BS = type 1 Brugada syndrome; EPS = electrophysiological study; ERS = early repolarization syndrome; ERS(A) = inferolateral ER combined with non-type 1 anterior ER; ERS(B) = pure inferolateral ER; FH = family history; ICD = implantable cardioverter-defibrillator; inf = inferior lead; IVF = idiopathic ventricular fibrillation of unknown cause; lat = lateral lead; SCA = sudden cardiac arrest; SCD = sudden cardiac death at <45 years of age; VF = ventricular fibrillation.