Homozygous Premature Truncation of the HERG Protein
The Human HERG Knockout

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Background—In long-QT syndrome (LQTS), heterozygosity for a mutation in 1 of the K\textsuperscript{+} channel genes leads to prolongation of the cardiac action potential, because the aberrant protein exhibits “loss of function.” HERG, which is involved in LQT2, is the gene encoding the rapid component of the delayed rectifier, I\textsubscript{Kr}.

Methods and Results—In a consanguineous family, a stillbirth was followed by the premature birth of a child in distress due to ventricular arrhythmia in the presence of QT prolongation. LQTS was diagnosed, \(\beta\)-blocker therapy was begun, and a pacemaker was implanted. She developed well and remained symptom-free for 1.5 years. In the index patient, we identified a duplication of bp 558 to 600 in exon 4 of HERG on both alleles. This will result in a frameshift and a premature stop codon before the S1 domain of the HERG protein. Because it is present on both alleles, no functional I\textsubscript{Kr} is anticipated. The same mutation was found heterozygously in both parents and homozygously in the stillborn brother.

Conclusions—It is concluded that absence of I\textsubscript{Kr} gives rise to a severe cardiac phenotype, with no indication of malfunction of any other organ. (Circulation. 1999;100:1264-1267.)

Key Words: genetics \(\bullet\) torsade de pointes \(\bullet\) arrhythmia

Congenital long-QT syndrome (LQTS) is an inherited disorder characterized by prolongation of the QT interval and the occurrence of polymorphic ventricular arrhythmia leading to recurrent (pre-) syncope or sudden cardiac death. The molecular basis of LQTS is heterogeneous, involving either inadequate opening of K\textsuperscript{+} channels (LQT types 1, 2, 5, or 6) or inadequate closing of Na\textsuperscript{+} channels (type 3).\textsuperscript{1-5} Heterozygosity for a mutation in 1 of the 4 K\textsuperscript{+} channel genes (LQT1, 2, 5, or 6) gives rise to prolongation of the cardiac action potential, because the aberrant protein encoded by the mutated gene exhibits “loss of function” (ie, less repolarizing K\textsuperscript{+} current, for example, by a dominant negative effect on channel function).\textsuperscript{2,6,7} This explains the autosomal-dominant inheritance pattern of Romano-Ward syndrome. Heterozygosity for mutations in KVLQT1 and KCNE1 have been associated with the recessively inherited Jervell and Lange-Nielsen syndrome, in which QT prolongation is associated with bilateral sensorineural deafness.\textsuperscript{8} Recently, a recessively inherited pattern of LQT1 without deafness was described.\textsuperscript{9} HERG, which is involved in LQT2, is the gene encoding the rapid component of the delayed rectifier I\textsubscript{Kr}.\textsuperscript{6} In this report, we describe a family in which consanguinity of the parents led to a homozygous mutation in HERG, putatively leading to a “human HERG knockout” in their offspring.

Clinical Data
The study was performed according to a protocol approved by the local ethics committee. The patients studied constitute the offspring of asymptomatic, consanguineous, Mediterranean parents (see inlay in Figure 1 for pedigree). After the birth of a healthy daughter (II-1, now 5 years of age), 1 child died in utero at 36 weeks of gestation with intrauterine heart failure and fetal hydrops (II-2). The index patient (II-3) was followed during gestation, and she presented in utero with an irregular heartbeat and a structurally normal heart.

Mutation Analysis
DNA was isolated from lymphocytes or amniotic fluid cells and DNA was extracted from the paraffin-embedded tissues of the stillborn child using the QIAamp DNA blood minikit (Qiagen). All 15 exons of the HERG gene were amplified from genomic DNA by the polymerase chain reaction (PCR) primers described.\textsuperscript{10} PCR products were analyzed by single-strand conformation polymorphism on 12.5% nondenaturating polyacrylamide gels run at 5°C and 15°C, as described in GeneExcel protocols (Pharmacia Biotech). DNA was stained using a silver staining kit (Pharmacia Biotech). DNA was stained using a silver staining kit (Pharmacia Biotech). Fragments presenting aberrant conformers were sequenced by the dideoxy chain termination method on an ABI 310 automatic sequencer (Applied Biosystems). Segregation of the 43-bp duplication in exon 4 in the family was determined by an analysis of the exon 4A PCR product on a 2% agarose/EtBr gel. A total of 100 control individuals were tested for the presence of the mutation.
At necropsy of the stillborn child, a normotrophic heart was diagnosed macroscopically. Paraffin blocks containing 4 full-thickness segments of the left (n=3) and right ventricular wall (n=1) were obtained. The 5-mm sections were stained with hematoxylin and eosin, elastic van Gieson, and van Kossa stains, respectively.

**Results**

**Patients**

The index patient (II-3) was examined shortly after birth. She had no external dysmorphic features (weight, 2.1 kg; length, 46 cm). All pulses were palpable. Heart rate was irregular. The heart was structurally normal on echocardiography, with moderate ventricular function.

The ECG obtained shortly after birth showed a relative sinus bradycardia (120/min), a PR interval and a QRS width of normally conducted impulses of, respectively, 120 and 60 ms, and a right axis (Figure 1). There was 2:1 functional atrioventricular (AV) block, with multiformal ventricular ectopy arising from the grossly abnormal TU complex. The QTc interval was 580 ms. Ventricular arrhythmias of the torsade de pointes type were observed, and the child deteriorated rapidly. Treatment with oral propranolol was instituted, leading to a significant reduction of ventricular arrhythmia. On day 2, polymorphic ventricular tachycardia was successfully terminated with intravenous magnesium sulfate (0.4 mmol/kg). Because 2:1 AV block persisted in the presence of β-blockade, permanent transvenous ventricular pacing was established on day 3, with a lowest rate setting of 100 beats/min. β-Blocker therapy was continued. Auditory evoked-response testing performed on day 7 of life showed a normal response at 70, 60, and 50 dB. The girl remained symptom-free at a follow-up of 1.5 years. She remains 100% paced.

Subject II-2 died in utero at 36 weeks of gestation with intrauterine heart failure and fetal hydrops. Heart weight recorded at autopsy was 15 g (normal weight is 16±4.3 g at 36 weeks of gestation). Histologic examination of the heart showed diffuse endocardial fibroelastosis in all sections (Figure 2). The myocardium showed thin, stretched bundles of myocardial cells partially arranged in a wavy pattern. Multifocal areas of calcification and extracellular myocardial calcium deposition were observed subendoocardially in the left ventricular wall (Figure 2). Moreover, distinct areas of coagulative necrosis were present. Inborn errors of metabolism were excluded as a cause of the cardiomyopathy.

The eldest daughter (II-1) was in good health at 5 years of age. Her ECG was normal, with a morphologically normal ST segment (QTc, 415 ms). Both the father (I-1, 28 years of age) and the mother (I-2, 24 years of age) had morphologically abnormal T-waves of slightly prolonged duration (QTc, respectively, was 440 and 450 ms). They never experienced any symptom compatible with LQTS. In their relatively large families (10 siblings and 16 children, respectively), with the exception of 1 stillbirth (details unknown), no individual was
symptomatic or died prematurely. All grandparents were alive and, apparently, in good health.

**HERG Analysis**

We identified a duplication of bp 558 to 600 in exon 4 of HERG, which resulted in a frameshift and a premature stop codon before the putative S1 domain, that eliminates the transmembrane domain of the protein. In the index patient, this duplication was present on both alleles, so no functional HERG protein is anticipated. The same mutation was found heterozygously in both parents; it was not present in the healthy sister or in 100 control individuals. Analysis of DNA isolated from amniotic fluid cells and paraffin-embedded tissues derived from the stillborn brother revealed that he also carried the duplication homozygously.

**Discussion**

HERG is the gene encoding I\textsubscript{Kr}, the rapid component of the delayed rectifier. Heterozygous missense mutations and mutations giving rise to premature translation stops are causally involved in LQTS\textsuperscript{2}.\textsuperscript{1} The HERG mutation described in the present family is expected to lead to premature truncation of the protein (before the S1 domain). The parents are consanguineous, and both are heterozygous for the mutation. The phenotype of the heterozygous state is apparently mild, with slightly abnormal QT intervals in both parents morphologically. In addition, the parents and their siblings are asymptomatic, and no suspected premature sudden cardiac death has occurred in 3 generations.

In contrast, homozygosity translated phenotypically into profound QT prolongation, with 2:1 (functional) AV block and severe ventricular arrhythmias before and immediately after birth in 1 child. This child was carefully followed in the prenatal period because a previous pregnancy ended with an in utero death at an estimated gestational age of 36 weeks. The boy, in whom homozygosity for the mutation could be demonstrated, died in a compensated state, as evidenced by the autopsy findings. A causal relation with the proven genetic abnormality can only be speculated, but it seems reasonable to suggest that severe ventricular arrhythmia was responsible for the intrauterine death, the more so because other causes of myocardial necrosis (ie, myocarditis and aberrant course of coronary arteries) could be excluded. The histopathological findings, ie, a markedly dilated heart with diffuse fibroelastosis, stretching of myocardial fiber bundles, areas of coagulative necrosis (recent ischemia), and multiple calcified scars (old infarction), agree with the suggestion of repetitive episodes of ischemia due to arrhythmia and provide an explanation for the cardiac failure and intrauterine death of the infant.

Clinical characteristics of LQTS with 2:1 functional AV block were recently reviewed.\textsuperscript{11} Twelve of 22 patients who were diagnosed in infancy died young.\textsuperscript{11} On clinical grounds, the majority of affected children were defined as “sporadic cases,” and de novo mutations were suggested.\textsuperscript{11} An alternative hypothesis is suggested by these cases, ie, the homozygous expression of HERG (or KVLQT1) mutations may lead to a severe phenotype. Indeed, it is becoming increasingly clear that LQTS, based on heterozygously expressed mutants, may appear with a very low penetrance.\textsuperscript{12}

As the mutation is expected to lead to premature truncation of the channel protein, homozygosity for the mutation will result in complete absence of I\textsubscript{Kr}. Interestingly, no other phenotypic expression seems to be present in our patients, suggesting a limited role of HERG in other human organ systems. In particular, no deafness is present, and psychomotor development appears normal. The (human) phenotype may, however, be lethal due to severe cardiac arrhythmias.

In conclusion, in humans, the absence of functional I\textsubscript{Kr} leads to a severe cardiac phenotype characterized by QT prolongation, functional AV conduction disturbances, and polymorphic ventricular arrhythmias. No evidence of dysfunction in any other organ exists.

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