Genetic basis of cardiac ion channel diseases

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Chapter 8

Long QT syndrome caused by a large duplication in the *KCNH2* (HERG) gene undetectable by the PCR-based exon-scanning methodologies employed to date

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Abstract

Background Studies carried out in large cohorts of Long QT syndrome (LQTS) probands have shown that in 30% of cases no mutation is identified in the LQTS-associated genes. Furthermore, the numerous mutations reported so far in these genes are point mutations or small insertions and deletions in coding regions or at splice junctions. However, the PCR-based exon-scanning methodologies employed routinely to date in mutation analysis are unable to detect large genomic alterations such as large deletions and duplications.
Objective The aim of this study was to test whether LQTS may be caused by large genomic alterations in the *KCNH2* or *KCNQ1* genes.
Methods Multiplex Ligation-Dependent Probe Amplification (MLPA) analysis, a quantitative multiplex approach to determine the relative copy number of gene exons, was used to screen a series of LQTS probands for large genomic alterations in the *KCNQ1* and *KCNH2* genes.
Results We identified the first large gene rearrangement consisting of a tandem duplication of 3.7 kb in *KCNH2*, responsible for LQTS in a Dutch family. All affected individuals were carriers of the duplication. This large duplication is expected to disrupt proper *KCNH2* pre-mRNA splicing, leading to non-functional or severely debilitated channels, decreasing I_{Kr} and thereby prolonging the QT-interval.
Conclusion Our findings carry implications for genetic testing in LQTS patients. Analysis for large gene alterations as the one described herein in routine genetic testing may provide a genetic diagnosis in a number of patients in whom conventional mutation screening fails to uncover a mutation.

Key words

Long QT syndrome, arrhythmia, ion channel, K^+ channel, genetics, mutation

8.1 Introduction

The Long QT syndrome (LQTS) is an inherited cardiac arrhythmia characterized by a prolonged QT interval on the surface electrocardiogram (ECG) associated with syncope and sudden death from *torsades de pointes* polymorphic ventricular tachycardia.[1] It is estimated to affect 1 per 5000 individuals.

LQTS may be caused by mutations in genes encoding K^+ channel pore-forming (*KCNQ1*, *KCNH2*) and ancillary (*KCNE1*, *KCNE2*) subunits and the gene encoding the cardiac Na^+ channel pore-forming subunit (*SCN5A*). Studies carried out in large cohorts of probands have shown that mutations in these genes are found in about 70% of cases.[2,3] However, the PCR-based exon-scanning methodologies (such as single-stranded conformation polymorphism (SSCP) analysis, denaturing high performance liquid chromatography (dHPLC) analysis, or direct sequencing of PCR-amplified coding regions) employed routinely to date in mutation screening of LQTS-associated genes are only able to identify point mutations or small insertions and deletions in coding regions or at splice junctions.[2,3] Due to the presence of the remaining normal allele, they do not detect large gene rearrangements such as large duplications and deletions (which may involve multiple exons), not-infrequent mutations in other disorders.[4] Thus, although yet-unknown genes could be involved in the remaining 30% mutation-negative patients, a number of cases might be attributable to large genomic rearrangements in these genes.

In this study we analyzed a series of mutation-negative LQTS probands for large genomic rearrangements in the *KCNQ1* and *KCNH2* genes by Multiplex Ligation-Dependent Probe Amplification (MLPA) analysis,[5] a quantitative multiplex approach to determine the relative copy number of gene exons. With this approach, we identified the first large gene rearrangement in *KCNH2* involving a duplication of 3.7 kb, responsible for LQTS in a Dutch family.

8.2 Methods

Patients

Twenty-one probands with a clear LQTS phenotype were ascertained at the Academic Medical Center, Amsterdam. The study was performed according to a protocol approved by the local ethics committee. Informed consent was obtained from the patients. Coding region or splice site mutations in the commonly involved LQTS-causing genes had been previously excluded in all probands by SSCP-DNA sequencing, dHPLC-DNA sequencing or by direct sequencing using primers in flanking intronic sequences.

MLPA analysis

Probes for MLPA analysis of *KCNH2* exons 1-4, 6, 9, 10 and 13 and *KCNQ1* exons 1-6 and 8-19 (exon numbering according to transcripts NM_000238 and NM_000218, respectively) were developed and manufactured for us by MRC Holland (Amsterdam) in close collaboration. The remaining exons of these genes were not probed since they are in very close proximity to those probed. In probe design, polymorphic sequences were avoided, because they could hamper hybridization and quantification. An additional 13 control probes for unlinked loci were also included. MLPA was carried out according to the manufacturer's instructions. In brief, 100 ng

DNA was denatured and hybridized overnight at 60°C with SALSA probe mix. After treating the samples with Ligase 65 for 15 min at 54°C, PCR amplification was performed with the specific SALSA FAM PCR primers. Electrophoresis of PCR products was done on an ABI PRISM 310. Data analysis was performed by exporting the peak-areas to a Microsoft Excel file. Sample-related and peak-related differences in PCR and electrophoresis efficiency were corrected by first calculating the peak-area relative to the sum of peak-areas per sample and subsequently expressing each normalized peak-area relative to the mean of that peak across samples. To detect deviating peaks, each such normalized peak was divided by the mean of that peak in the control samples. Peak heights outside the range 0.7 to 1.3 times the control peak height were considered abnormal, with those below 0.7 representing deletions and those above 1.3 representing duplications. Several control samples were included in each MPLA test. Each result was confirmed by two independent experiments.

Confirmation and analysis of the large duplication in KCNH2 gene by PCR

For confirmation of the duplication in *KCNH2* and to estimate the size of the duplicated fragment and locate its boundaries, PCR was performed using the LA-PCR method (TaKaRa), with the following primer combination: 5'-CTTCCGCACCACCTACGTCAATGCC-3' (Forward) and 5'-GGGCTGTAATGCAGGATGGTCCAGC-3' (Reverse). Both primers hybridize to sequences in exon 6, with the forward primer located 3' to the reverse. Thus, an amplification product is only possible in DNA of duplication carriers.

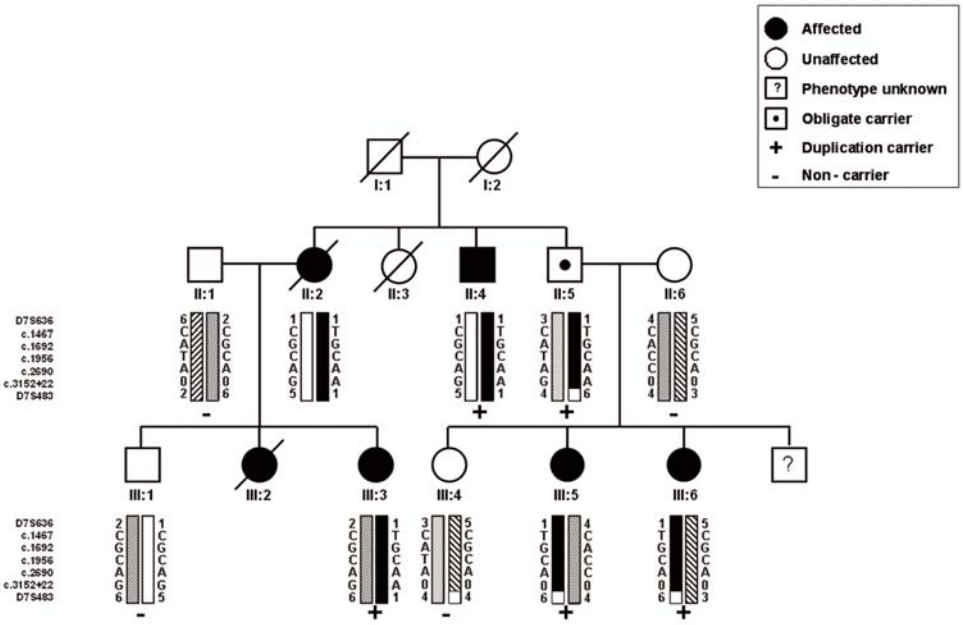

Figure 1: Pedigree of the family displaying *KCNH2* haplotypes. Haplotypes were constructed by genotyping gene-flanking microsatellite markers and intragenic polymorphisms. The allele represented in solid black contains the 3.7 kb-duplication. Individuals marked with a plus sign are carriers of the duplication. Individuals marked with a minus sign are non-carriers

8.3 Results

Detection of a large duplication in the KCNH2 gene

An aberrant exon copy number was detected in one proband (Figure 1, III:3). After quantification, the copy number of *KCNH2* exon 6 was estimated to be 3.0 (Figure 2A), indicating that the exon 6 on one of the alleles was duplicated. PCR amplification using the exon 6 primers described above confirmed this finding and revealed that the region duplicated was ~3.7 kb (Figure 2B). All affected family members were positive for this PCR product, while no PCR product resulted with DNA from control individuals (Figure 2B). DNA sequencing of this junction fragment revealed that the heterozygous duplication consisted of a tandem duplication of 3682 bp, from position c.1129-2543, and included 2.5 kb of intron 5, exon 6 (429 bp), intron 6 (589 bp) and the first 121 bp of exon 7 (Figure 2C). The duplication therefore disrupts exon 7.

Clinical data of the family with the large genomic duplication in KCNH2

The family history is characterized by a sudden unexpected nocturnal death of a 26 year old, previously syncopal female patient (III:2, see pedigree in Figure 1). Shortly after her death, the diagnosis LQTS was made in her younger sister (III:3), who was symptomatic from age 17 with, among others, acoustic triggers. The QT_c interval (Bazett's formula) was 0.48 s and a *torsades de pointes* arrhythmia could be evoked by an early morning wake-up call. Syncopal attacks triggered by acoustic stimuli were also reported by the mother (II:2) and for the deceased sister. A cousin (III:5) from the maternal side collapsed at age 27 after a period of diarrhea while on Mephaquin (malaria prophylaxis). QT_c was 0.48 s. One of her sisters (III:6) also had slightly prolonged QT-intervals. Subject II-4 is asymptomatic. No clinical data is available for subject II:3. All symptomatic patients are successfully treated (for many years) with β-blockers.

8.4 Discussion

Using MLPA analysis we identified a 3.7 kb-duplication in *KCNH2* underlying LQTS in a Dutch family. *KCNH2* encodes the α-subunit of the channel for the rapidly activating delayed rectifier K^+ current, I_{Kr}, a key player in cardiomyocyte repolarization.[6] Mutations in *KCNH2* prolong repolarization, and consequently the QT interval, by a loss-of-function mechanism. Outward K^+ current may be reduced by alterations in channel gating or kinetics, or by protein trafficking defects that reduce delivery of channels to the cell surface membrane.[7, 8] The identification of a *KCNH2* gene defect in this family is not surprising since acoustic triggers, observed in II:2, III:2 and III:3, occur predominantly in patients with a *KCNH2* genetic defect.[9]

The duplication identified in this family is expected to disrupt proper *KCNH2* pre-mRNA splicing with profound consequences on *KCNH2* protein structure, leading to non-functional or severely debilitated channels. Various scenarios may be envisaged. Naming only two possibilities, the disrupted exon 7 may be included in the transcript through cryptic splicing with retention of intronic sequences and introduction of a premature termination codon, or may be skipped resulting in a mRNA including a duplicated exon 6. Moreover, exon 7 encodes critical structural components of the channel protein (transmembrane segments S4, S5, and part of the pore loop) and disruption of this exon is therefore expected to have serious consequences on the channel protein structure.

The limited number of LQTS probands studied precludes the assessment of the prevalence of large gene duplications or deletions in LQTS. This needs to be assessed in future studies by centers with larger patient cohorts. While large gene rearrangements as the one we describe here might explain some of the LQTS cases, one should take into account that mutations in non-coding regions (such as introns or promoter regions) of known LQTS-associated genes, as well as mutations in yet unknown genes can also be responsible for the remaining 30% mutation-negative LQTS patients. Furthermore, similar genetic defects may occur in *KCNE1* or *KCNE2*, not tested in this study.

Although MLPA testing in *KCNQ1* and *KCNH2* is new, and aspects such as sensitivity, specificity and reproducibility of this test are yet to be determined, the importance of identification of the causative mutation to the individual patient and relatives, favor implementation of MLPA analysis in routine genetic testing of those individuals in whom a mutation has been excluded by the exon-scanning methodologies.

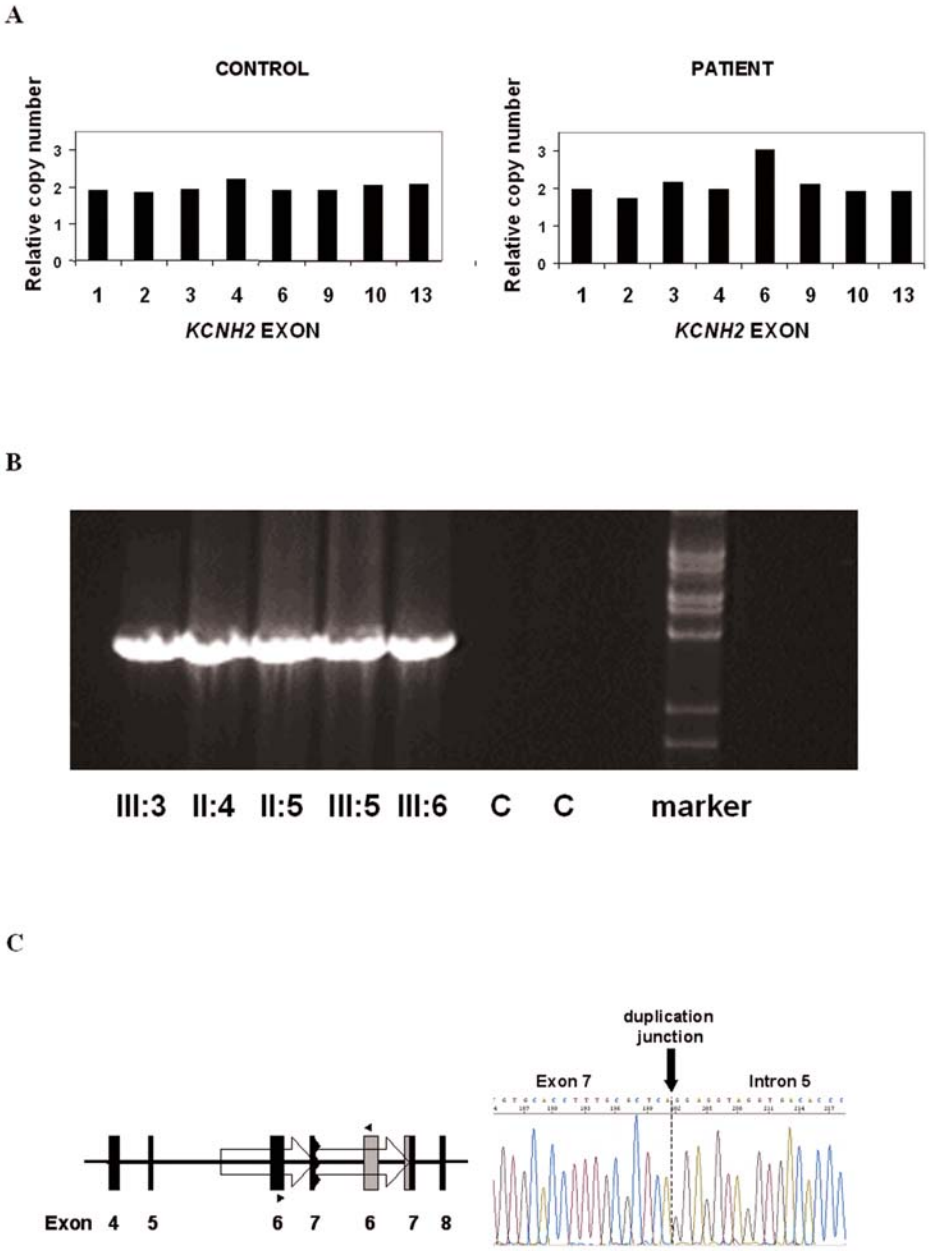

Figure 2: A: Relative copy number of *KCNH2* exons of a control individual (left panel) and of patient III:3 (right panel) determined by MLPA analysis. Note the increased copy number of exon 6 in the patient. **B**: PCR amplification confirmed the presence of the duplication and revealed that the region duplicated was ~3.7 kb. A PCR product was obtained with DNA from affected family members. No PCR product resulted with DNA from control (C) individuals. **C**: Diagrammatic representation of the *KCNH2* gene tandem duplication (left panel) and the DNA sequence across the duplication junction (right panel). The small arrow heads in the left panel indicate the location of the primers used for PCR analysis of the duplication.

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