Clinical and genetic spectrum of hereditary cardiac arrhythmia syndromes

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A Novel Early Onset Lethal Form of Catecholaminergic Polymorphic Ventricular Tachycardia Maps to Chromosome 7p14-p22

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Abstract

Introduction: Previously, autosomal dominant Catecholaminergic Polymorphic Ventricular Tachycardia [CPVT (1)] was mapped to chromosome 1q42-43 with identification of pathogenic mutations in RYR2. Autosomal recessive CPVT (2) was mapped to chromosome 1p13-21, leading to the identification of mutations in CASQ2. In this study, we aimed to elucidate clinical phenotypes of a new variant of CPVT (3) in an inbred Arab family and also delineate the chromosomal location of the gene causing CPVT (3).

Methods and Results: In a highly inbred family, clinical symptoms of CPVT appeared early in childhood (7-12 years) and in three of the four cases, the first appearance of symptoms turned into a fatal outcome. Parents of the affected children were first-degree cousins and without any symptoms. Segregation analysis suggested an autosomal recessive inheritance. Genome-wide search using polymorphic DNA markers mapped the disease locus to a 25-megabase interval on chromosome 7p14-p22. A maximal multipoint LOD score of 3.17 was obtained at marker D7S493. Sequencing of putative candidate genes, SP4, NPY, FKBP9, FKBP14, PDE1C, TBX20, in and around this locus, did not reveal any mutation.

Conclusions: We have identified a novel highly malignant autosomal recessive form of CPVT and mapped this disorder to a 25-megabase interval on chromosome 7p14-p22.

Key words: CPVT, Sudden Cardiac Death, Genetics, New Locus

Introduction

Significant percentages of Sudden Cardiac death (SCD) at or below the age of 40 yrs have a genetic background, mainly due to the Long QT syndrome (LQTS), Brugada syndrome, Conduction defects, Hypertrophic cardiomyopathy (HCM), Dilated cardiomyopathy (DCM), and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT).1-3 CPVT is one of the prevalent causes of SCD during childhood,4 and in adolescence.5 CPVT is an arrhythmogenic cardiac disorder characterized by reproducible form of ventricular tachycardia, induced by physical activity, stress or catecholamine infusion, which can deteriorate into ventricular fibrillation and syncope. Leenhardt A et al.4 and Eisenberg SJ et al.6 reported extensively on the clinical spectrum of CPVT patients. CPVT can be inherited in an autosomal dominant,7-10 or recessive way.11-13 Clinical cardiological examinations, ECG parameters, including the QTc interval are generally normal or borderline prolonged, however sinus bradycardia is frequently observed.10 Echocardiography generally reveals normal findings, and postmortem examinations, when carried out, do not reveal any significant morphological alterations in the fine structure of the heart, with the exception of mild fatty infiltration in a few patients.14,15 The clinical penetrance ranges from 25 to 100%, with an average of 70-80%. Syncope appears to be the first symptom in more than half of the
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patients and occurs in a wide age range between 3-51 yrs.\textsuperscript{4,10,16} When untreated, the mortality of CPVT is high, reaching 30-50\% by the age of 20-30 years.\textsuperscript{16} \(\beta\)-blockers without sympathomimetic activity are clinically effective in reducing syncope,\textsuperscript{8,10} but implantation of defibrillator was occasionally needed in some patients.\textsuperscript{9}

The gene locus corresponding for autosomal dominantly inherited CPVT (1) was initially mapped to chromosome 1q42-43,\textsuperscript{7} thereafter several groups reported various missense mutations in the \textit{RYR2} gene located in this locus.\textsuperscript{8-10,17,18} Identification of a recessive locus for CPVT (2) on chromosome 1p13-21,\textsuperscript{11} led to identification of pathogenic mutations in \textit{CASQ2}.\textsuperscript{12,13,19}

In the present study, we describe the identification of a new malignant form of CPVT (3) in four closely related patients, inherited as an autosomal recessive trait with full penetrance during childhood. We have performed homozygosity mapping in this highly inbred family to determine the region. A novel locus for this malignant CPVT subtype maps to chromosome 7p14-p22. Furthermore, we have screened positional candidate genes present in the segregating and in the adjacent locus.

\section*{Methods}

\textbf{Clinical Evaluation of the proband and the family members}

The patients studied constitute the offspring of asymptomatic, consanguineous, Sudanese parents (Figure 2A), living in the United Arab Emirates (UAE). Clinical evaluation was done at the department of Pediatrics, Tawam Hospital of UAE University. Informed consent was obtained from the participants or their guardians in accordance with the regulations. Phenotyping was carried out on the basis of clinical history, physical examinations, resting and exercise induced ECGs, and echocardiography. Due to access/communication limitations, the cousins, aunts and other extended family members could not be included in this study, however no history of sudden cardiac death in the extended family members was reported.

\textbf{Genetic Analysis}

Genetic investigations were performed at the department of Clinical Genetics at the Academic Medical Centre of the University of Amsterdam. Genomic DNA was isolated from peripheral blood lymphocytes according to established protocols. Bi-directional DNA sequencing of the entire coding exons and intro-exon junctions of \textit{RYR2}, \textit{CASQ2}, \textit{KCNJ2}, \textit{FKBP12.6}, \textit{SCN5A}, \textit{KCNH2}, \textit{KCNQ1}, \textit{KCNE1}, \textit{KCNE2}, \textit{KCNJ2} and \textit{NCX1} was performed according to established protocols in our laboratory. Considering the consanguinity of the
parents, and the absence of sudden death at a young age among the immediate prior generation family members, we initially also performed homozygosity mapping around candidate genes \textit{RYR2, CASQ2, SCN5A, KCNH2, KCNQ1, CLCN2, ANK-B, CALNA2, KIR6.2, CACNA1C, KCNJ2, KCNE1, KCNE2,} and \textit{KCNJ4}, in which mutations were reported to cause electrical instability of the heart.\textsuperscript{1-3} Homozygosity mapping utilises the concept that affected individuals will be homozygous by descent for a mutation and polymorphic markers nearby. Microsatellite repeat markers from ABI-Prism Linkage Mapping Set encompassing these genes were used. Subsequently, a genome-wide scan was performed using the remaining polymorphic markers from chromosomes 1 to 22. These markers span the human genome with an average spanning/interval of 10 cM. Fine mapping was performed using additional markers \textit{D7S481, D7S526, D7S2557} and \textit{D7S493}, identified at the GDB database. The genotyping was performed using an ABI 3100 Genetic Analyzer. Moreover, the entire coding exons of \textit{SP4 (exons 1-6), FKBPs9 (exons 1-10), FKBPs14 (exons 1-4), PDEIC (exons 1-19), NPY (exons 2-4), TBX20 (exons 1-6)} and their exon-intron junctions were screened for mutations. Primer sequences and PCR conditions are available on request.

**Linkage Analysis**

Phenotype- and genotype data and pedigree information were combined for multipoint linkage analysis with the use of the easyLinkage v5.02 software package.\textsuperscript{20} From this multipoint linkage analysis was performed running the Merlin program v1.0.1,\textsuperscript{21} with the assumption of an autosomal recessive pattern of inheritance, a disease-allele frequency of 0.0001, and penetrance of 0 for carriers and noncarriers and 0.99 for homozygous affected individuals. Gene frequency was assumed to be equal between males and females. The distance (cM) between markers was obtained from the Marshfield map.\textsuperscript{22}

**Results**

**Clinical analysis**

3 children with an average age of 10±2 years died suddenly during playing (see below), one surviving male child (IV:9) had his first syncope and seizures at the age of 7 years. Subject IV:1: Her initial presentation was sudden death at the age of 10 years, while playing in a fun park. Resuscitation was unsuccessful, and she died before any work up could be completed. Subject IV:2: Collapsed at 12 years while skating in the mall. During resuscitation, he developed several episodes of VT. ECGs taken in the hospital after collapse showed mildly prolonged QTc (450-490 ms) together with documented premature ventricular contrac-
tions (PVC’s) during and after resuscitation, unrelated to electrolyte problems. The PVC’s had RBBB morphology, with superior axis, indicating LV origin. He was on β-blockers (Propanolol 20 mg PO TID) until death. He remained in almost vegetative state until death at 14 years (vegetative state for 2 years) because of respiratory failure, due to pneumonia. No recurrence of VT was observed during that time. An echocardiogram did not reveal any structural anomaly of the heart. No post-mortem studies were performed on him or in any of his siblings who expired. Subject IV:4: Diagnosed at birth with severe form of tetralogy of Fallot (pulmonary atresia/ VSD). She underwent first palliative cardiac surgery as a neonate (Blalock-Taussig shunt). Baseline ECG before surgery (at 3d) showed QTc = 440 ms. QTc at age 14 months was 480-490 ms. A second (corrective) cardiac surgery occurred at 3 years. The postoperative ECG showed RBBB with QRS duration of 110 ms and QTc=470-490 ms (not corrected for widened QRS complex). She collapsed during sports at 8 years, and died immediately. Subject IV:9: The index patient is presently 7 years old, he is the only surviving patient. He presented to the ER after syncope occurred while playing in the backyard and was resuscitated promptly by an uncle. ECG showed borderline prolonged QTc= 480 ms (Figure 1A), while echocardiogram revealed normal findings. 24-hour Holter monitoring and an exercise stress test showed borderline prolongation of QTc (470-480 ms) (Figure 1B). Isolated PVC’s occurred at a heart rate (HR) of 144 bpm, couplets appeared at a HR of 170 bpm (Figure 1B) after exercising for 6 min, subsequently the test was terminated. No hypotension or symptoms were reported. Ventricular arrhythmia disappeared.

Figure 1A: Resting ECG of patient IV:9
when the HR was <144 bpm during recovery. He has been put on β-blocker (Metoprolol 50 mg PO OD) and has had no further symptoms, and is restricted from exercise for last 18 months.

Amongst the other family members no significant clinical findings were present, except for mother III:2 who had two miscarriages, all in the first or second trimester (first miscarriage occurred after IV:2, and the second after IV:5, both are not shown in the pedigree). Siblings IV:5 to IV:7, had no signs of PVC upon 24-hrs Holter monitoring. The resting and exercise ECGs of father III:3 are normal, and mother III:4 recently delivered a healthy male newborn, who has normal resting ECG, and normal echocardiogram. Her resting ECG is also normal. Exercise ECG or Holter monitoring could not be performed in the other siblings (IV:8, IV:10 and IV:11) or in the parents of IV:9, though their base line ECG is normal, and they have no history of syncope.

**Genetic Analysis**

Karyotype analysis excluded any chromosomal aberration in IV:9. This study was performed in several phases spanning over 3-years. The family initially came to our attention when IV:2 (Figure 2) had a syncopal attack at the age of 12 yr, and his immediate older sister IV:1 had already died at 10 yrs. Direct DNA sequencing of genes that are involved in electric impulse propagation of the heart (RYR2, CASQ2, KCNJ2, FKBP12.6, SCN5A, KCNH2, KCNQ1, KCNE1, KCNE2, KCNJ2, NCX1) did not reveal any mutation. Addition-
ally, sequencing of the putative promoter region and also genomic deletion analysis for RYR2 did not reveal any deletion (data not shown).

As all affected children were the progeny of consanguineous unaffected parents, we initiated homozygosity mapping around the known CPVT causing genes, RYR2 and CASQ2, table-1 shows the unlinked haplotypes of the microsatellite repeats encompassing these gene in the affected children. Similarly, homozygosity mapping around other genes reported to cause electrical instability of the heart, did not produce any shared haplotypes among the affected subjects (data not shown). Subsequently, whole genome scanning revealed a homozygous region on chromosome 7p14-p22 (Figure 2), shared between IV:2 and IV:4.

**Figure 2:** Pedigree of the consanguineous family studied. Affected individuals are shown as filled circles (females) and squares (males). Normal individuals are depicted with empty symbols, and deceased individuals are indicated by slashes. The proband is indicated by an arrow. Double lines indicate consanguineous marriages. The region of homozygosity is demarcated by the box.

**Table 1:** Haplotype analysis of the four CPVT affected children with microsatellite repeat markers encompassing the RYR2 and CASQ2. No shared haplotype/s were detected among the affected children. Location of the genes (CASQ2, RYR2) are shown between the markers.
Further, additional mapping with more closely spaced markers showed consistent inheritance of markers identical by descent (IBD) and was still observed in the affected subjects (Figure 2). This homozygous region is distally flanked by the D7S526 marker and proximally by D7S481. This is the only homozygous region which shows complete co-segregation with the disease phenotype, while it is absent from the non-symptomatic children or a sibling with Pulmonary Stenosis (PS) and Ventricular Septal Defect (PSD) (IV:8). A maximal multipoint LOD score of 3.17 was obtained with marker D7S493 (Figure 3).

**Candidate Gene Screening in the Linked Recessive locus**

The 25-Mb interval between D7S526 and D7S481 contains 172 expressed sequence tags (ESTs), 7 of them are known cardiac expressed genes (*SP4*, *DNAH11*, *PDE1C*, *FKBP4, FKBP9, NPY* and *TBX20*) (Figure 4). SP4 was one of the most plausible candidate gene, mice deficient for SP4 were reported to have sudden cardiac death without any cardiac structural aberration. Another gene is *NPY*, which exerts an effect on maturation of L-type Ca2+ during postnatal development in vivo. Four additional cardiac expressed genes *PDE1C*, *FKBP9*, *FKBP14* and *TBX20*, (Figure 4) located in the immediate out-skirt of the linked locus, were also screened. However, no significant sequence alterations were detected in coding exons or the exon-intron boundaries in any of the screened genes.
Discussion and Conclusion

Our study demonstrates a new malignant variant of CPVT (with minor QT-prolongation) in an inbred family with autosomal recessive inheritance. No mutation was detected in any of the genes, RYR2, CASQ2, KCNJ2, FKBP12.6, SCN5A, KCNH2, KCNQ1, KCNE1, KCNE2, KCNJ2 and NCX1 which are all involved in the electrical impulse propagation of the heart. We have mapped this autosomal recessive CPVT (3) locus to chromosome 7p14-p22. Recombinant analysis placed the homozygous region between markers D7S526 and D7S481, a region spanning 25 Mb. No other genetic disease/s has been reported to be linked in this locus, but, Song L et al. recently identified the adjacent locus 7p12-q21 in a new variant of autosomal dominant cardiomyopathy, but no gene could be identified in their study. The CPVT phenotype in this family appears to be severe, because it is manifested by onset at an early age (mean age ±10 yrs) and is associated with sudden death during childhood (range 8-12 year). In concordance with a recessive phenotype, neither the heterozygous carriers/parents or brother (IV:6) showed any signs of PVC or CPVT. Onset of CPVT(1) symptoms are reported as early at 3 yrs age but can be as late as 51 yrs. Many patients survive multiple syncopal episodes, but mortality rate without treatment is 30-50% by the age of 20-30 yrs. Till now a total of 6 mutations, either homozygotes or compound heterozygotes in CASQ2 are reported in CPVT(2) patients. In all three studies, onset of symptoms was before the age of 7 yrs and by the age of 10 yrs the disease was fully penetrant. Though the number of affected children is small in our study (n= 4), the onset and severity of symptoms are comparable to the bi-allelic CASQ2 mutation carriers. Both RYR2 and CASQ2 mutation carriers were reported to have normal to borderline prolonged QTc, which was also observed in our affected subjects (average QTc= 470 ms). Typical onset of syncope in our studied family is 10±3 years, and fatal at the first syncopal attack in three out of four cases.

The homozygous region shared by the affected family members is a 25-Mb interval between markers D7S526 and D7S481, it contains 172 genes of which 7 are known to be cardiac expressed (SP4, DNAH11, PDE1C, FKBP4, FKBP9, NPY and TBX20). SP4 was one of the most plausible candidate genes, as mice deficient for SP4 survive to term, exhibit normal cardiac structure and function, but display sudden cardiac death and severe conduction system defects, including spontaneous ventricular arrhythmias (Nguyen-Tran van TB et al. 2000). We have also screened NPY, which is expressed in neurons of the intracardiac ganglia and exerts an effect on maturation of L-type Ca2+ during postnatal development in vivo. Four additional genes located in the immediate vicinity of 25-Mb interval, all expressed in human heart, were also screened. Among them, PDE1C, which functions as a Ca+2/calmodulin dependent cAMP and cGMP phosphodiesterase, possibly playing a role signal transduction, and FKBP9 and FKBP14 which are involved in calcium ion
handling. However, we did not check DNAH11 for mutations, although it is expressed in the heart, DNAH11 mutations cause situs inversus totalis in combination with primary ciliary dyskinesia phenotypes, which were absent from our patients. We could not detect any significant sequence alterations throughout the coding region in any of the screened genes, though it is possible that pathogenic sequence alterations lie in areas we didn’t investigate such as regulatory regions, like promoters and intronic sequences. Our patients don’t have homozygous deletions in either SP4 or NPY as we could always amplify the exons and the exon-intron junctions, which precluded us in performing MLPA based deletion/duplication analyses. Of the 172 expressed sequence tags (ESTs) confined to this region, 7 currently show expression in cardiac tissue. It remains well possible that the pathogenic mutation might lie in any of the yet undefined ESTs.

Though our number of studied subjects is small, it led us to the identification of a second recessive locus for CPVT. Once the gene is isolated responsible for this particular phenotype, it could possibly lead to development of a successful treatment strategy in preventing unwanted premature SCD.

![3](image)

### Study Limitations

**Major difficulty** encountered in the present study was our inability to perform exercise ECG in IV:10, IV:11 and their parents (III:3 and III:4), similarly in III:2, IV:3, IV:5, IV:6, IV:7, IV:8 and their mother (III:2). Despite the mentioned people have no expressed clinical

<table>
<thead>
<tr>
<th>No.</th>
<th>(Pedigree Reference)</th>
<th>Sex</th>
<th>Age at first syncope (yrs)</th>
<th>Precipitating factor for syncope</th>
<th>Age of death (yrs)</th>
<th>Resting heart rate (bmp)</th>
<th>QTc (sec)</th>
<th>PVT Threshold (lpm)</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>IV:1</td>
<td>F</td>
<td>10 yrs</td>
<td>Exercise</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>None</td>
</tr>
<tr>
<td>2.</td>
<td>IV:2</td>
<td>M</td>
<td>12 yrs</td>
<td>Exercise</td>
<td>64 yrs</td>
<td>450-450 ms</td>
<td>PVC's seen after resuscitation at 180-140</td>
<td>-5-Rockets after arrest (Procainamide) until he died</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>IV:4</td>
<td>F</td>
<td>8 yrs</td>
<td>Exercise</td>
<td>96</td>
<td>450-450 ms (before surgery)</td>
<td>NA</td>
<td>None</td>
<td>None (had surgical repair of PA/VSD)</td>
</tr>
<tr>
<td>4.</td>
<td>IV:9</td>
<td>M</td>
<td>7 yrs</td>
<td>Exercise</td>
<td>82</td>
<td>450 ms</td>
<td>155; Isolated PFC's 1/26; Coumadin</td>
<td>-5-Rockets (Metoprolol)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Clinical data on patients with CPVT
phenotype/s which suggests CPVT3 as autosomal recessive inheritance, exercise ECG in these clinically silent family members might have given more information in the carriers of one putative pathogenic allele. In this study, patients with CPVT3 had also borderly prolonged QT intervals. Though, we assume that this is an added phenotype combined with CPVT. But, exercise ECG in the non-symptomatic family members would have elucidated more about this phenomenon in the non-carriers and mono-alleleic carriers.

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