Autosomal Recessive Catecholamine- or Exercise-Induced Polymorphic Ventricular Tachycardia

Clinical Features and Assignment of the Disease Gene to Chromosome 1p13-21

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Background—Catecholaminergic polymorphic ventricular tachycardia (PVT) is characterized by episodes of syncope, seizures, or sudden death in response to physiological or emotional stress. In 2 families with autosomal dominant inheritance, the disease gene was mapped to chromosome 1q42-43. The objectives of this study were to characterize the clinical features of the disease in a Bedouin tribe from Israel and to map the disease gene.

Methods and Results—In this Bedouin tribe, 9 children (age, 7±4 years) from 7 related families have died suddenly during the past decade, and 12 other children suffered from recurrent syncope and seizures starting at the age of 6±3 years. Parents of affected individuals were asymptomatic and were all related (first-, second-, or third-degree cousins). Segregation analysis suggested autosomal recessive inheritance. All 12 symptomatic patients and 1 asymptomatic sibling (mean age, 13±7 years) were found to have a relative resting bradycardia (64±13 bpm, versus 93±12 bpm in the unaffected siblings), as well as PVT induced by treadmill or isoproterenol infusion and appearing at a mean sinus rate of 110±10 bpm. Patients responded favorably to treatment with β-blockers. A genome-wide search using polymorphic DNA markers mapped the disease locus to a 16-megabase interval on chromosome 1p13-21. A maximal lod score of 8.24 was obtained with D1S189 at θ=0.00. Sequencing of KCND3, a gene that encodes an Ito potassium channel transporter, did not reveal any significant sequence alterations.

Conclusions—This unique form of autosomal recessive PVT affects young children and may be lethal if left untreated. Linkage analysis maps this disorder to chromosome 1p13-21. (Circulation. 2001;103:2822-2827.)

Key Words: tachycardia ■ genetics ■ mapping ■ death, sudden ■ syncope

Sudden death due to cardiac arrhythmia is a devastating event, especially when it occurs in children. In the absence of structural heart defects, 5 major arrhythmogenic disorders manifesting as polymorphic ventricular tachycardia (PVT) or ventricular fibrillation have been described: (1) the long-QT syndrome, (2) right bundle-branch block and persistent ST elevations (Brugada syndrome), (3) the short-coupled variant of torsade de pointes, (4) idiopathic ventricular fibrillation with normal ECG, and (5) PVT induced by catecholamines. The last entity was initially described as a case report in 1975, as a short series in 1978, and as a distinct clinical entity in 1995. Leenhardt et al described 21 children who had been referred because of stress- or emotion-induced syncope, who did not have structural heart disease and had a normal QT interval. The hallmark of the disease was a reproducible form of PVT without QT-interval prolongation, which appeared during exercise test, isoproterenol infusion, or other forms of adrenergic stimulation and could degenerate to ventricular fibrillation. The average age at the onset of symptoms was 7.8±4 years, and treating these children with β-blockers alleviated their symptoms and the episodes of PVT. In 7 patients, there was a family history of syncope or sudden death, which suggests a genetic predisposition, though no clear pattern of inheritance was reported.

Recently, Swan et al evaluated 2 unrelated families with an inherited cardiac syndrome causing stress-induced PVT,
seggregating in an autosomal dominant mode. Symptoms in these 2 families appeared at an average age of 21±10 years, and among affected family members the cumulative cardiac mortality by the age of 30 years was 31%. Affected members in these families were also successfully treated with β-blockers. Using linkage analysis, Swan et al5 mapped the disease in these 2 families to chromosome 1q42-43. Priori et al6 identified 4 missense mutations in the ryanodine receptor 2 gene (1q42) in families suffering from this disorder.

In this report, we describe a unique form of autosomal recessive catecholamine- or exercise-induced PVT found in a highly inbred Bedouin tribe from the north of Israel. We also present the results of a genome-wide search that maps this disorder to the short arm of chromosome 1.

Methods

Families and DNA Extraction

Families were recruited at the Tel Hashomer and Rambam Medical Centers in Tel Hashomer and Haifa, Israel. The institutional review board at each center approved the study and participants gave informed consent. Each of the 41 living family members who participated in this study had 20 mL of heparinized blood drawn (Figure 1), and DNA was extracted with a commercial kit (PureGene, Gentra systems). In addition, we extracted DNA from a frozen blood sample of 1 child who died a few months before the initiation of the study and who had had symptoms identical to those of the other patients. In families 1, 2, 3, and 7, the parents are first cousins. More distant relationships between the parents could be identified in the other families.

All living family members (n=41) had a physical examination, 12-lead ECG, 2D echocardiography, treadmill exercise testing, and
holter monitoring. Isoproterenol infusion was used in 2 individuals. QTc was calculated according to Bazett’s formula.7 QT intervals were measured in lead II and, if possible, in lead III and AVF, from the onset of the QRS complex to the end of the T wave (defined as the return of the T wave to the isoelectric baseline or its extrapolation to the baseline). All living family members were defined as affected on the basis of the induction of PVT by an exercise test or by isoproterenol infusion.

Genotyping and Sequencing
A genome-wide search was performed using the Weber 8.0 screening kit (Research Genetics), which contains 387 di-, tri- and tetranucleotide repeat markers evenly spaced throughout the genome. Markers described in this study included D1S2849, D1S2868, D1S3723, D1S187, D1S418, D1S189, D1S2784, D1S534, and D1S514. Amplification was carried out in a 10-µL reaction volume containing 50 ng of DNA, 13.4 ng of each unlabeled primer, 1.5 mmol/L deoxynucleotide triphosphates, 0.08 µg 32P-labeled primer in 1.5 mmol/L MgCl2, polymerase chain reaction buffer, with 1.2 U of Taq polymerase (Bio-Line, London). After an initial denaturation of 5 minutes at 95°C, 31 cycles were performed (94°C for 2 minutes, 52°C for 3 minutes, and 72°C for 1 minute), followed by a final extension of 7 minutes at 72°C. Samples were mixed with 10 µL of loading buffer, denatured at 95°C for 5 minutes and electrophoresed on a 6% denaturing polyacrylamide gel.

Exons 1 to 7 of the KCND3 gene were amplified using the conditions described above and the following intronic primers: 5'-ATGAAATACAGTGAATGATTGG-3' and 5'-GCCACCAGCTTTTTACTCAATC-3' for exon 1; 5'-ATCCTCTGCTCTGTTCTT-3' and 5'-GCTCCCCGCATCCTTTTACTCTG-3' for exon 2; 5'-TCCTCCCTGATCCTAC-3' and 5'-GCCACCACAGCTCTCATT-3' for exon 3; 5'-GCCACGCACTCTGAGT-3' and 5'-GCCACCACAGCTCTTCATT-3' for exon 4; 5'-CAATCCAATGGGTCAGGTCA-3' and 5'-GCCAGCACTCTCATT-3' for exon 5; 5'-TCTCTTACACCTTTTCTACT-3' and 5'-TGCTGGTGTGCCTAG-3' for exon 6; and 5'-TATTAGAAAGGTCAGGTCA-3' and 5'-GCCACGCACTCTCATT-3' for exon 7. Amplification products were sequenced using an ABI Prism-310 Genetic Analyzer (Perkin-Elmer).

Linkage and Haplotype Analysis
Linkage was calculated with the LINKAGE (version 5.1) package of computer programs,8 assuming an autosomal recessive model of inheritance, 100% penetrance in both sexes, and a gene frequency of 0.001. Equal allele marker frequencies were assumed. The marker order and distance, taken from the Unified Database for Human Genome Mapping,9 is shown in Figure 2. Haplotypes were inferred so as to minimize recombinants.

Statistics
Comparisons between groups were performed with 2-tailed Student’s t test for normally distributed parameters. Data are expressed as mean±SD. P<0.05 was considered significant.

Results
Patient Characterization
We have identified 7 families, all from the same Bedouin tribe in the north of Israel, in which 9 children have died suddenly during the last decade. Of these, 7 deaths occurred during vigorous exercise and 2 during excitement. In addition, 12 other individuals suffered from recurrent episodes of syncope and seizures. Approximately 70% of these episodes appeared during vigorous physical activity, and 30% followed sudden excitement. The patients’ clinical features are presented in Table 1. All had a normal physical examination and a normal ECG. The average age at the onset of symptoms was 6±3 years. One patient, a 7-year-old boy, was asymptomatic but was diagnosed during a treadmill test.

All patients had PVT induced by treadmill or isoproterenol infusion (Figure 3). The duration and number of sequential beats of the tachycardia varied considerably. The tests were stopped once arrhythmia was detected, and therefore, the significance of the number and duration of ventricular tachycardia (VT) is unclear. In some patients, only short runs of PVT were noted, whereas in others, continuous PVT lasted for >60 s. The VTs detected in the patients were similar to those described as polymorphic by Leenhardt et al.4 We have not seen any typical bidirectional VT. The arrhythmia was always reproducible by exercise or isoproterenol test. None of our patients has undergone electrophysiological study because it was considered clinically noncontributory. The average QTc of the patients (before initiation of treatment) was normal, 0.4±0.02 s (range, 0.37 to 0.43 s) compared with 0.37±0.016 s (range, 0.36 to 0.41 s) in the unaffected siblings (P<0.002). The patients had a relative resting bradycardia, 64±13 bpm compared with 93±12 bpm in the unaffected siblings (P<0.002). The average heart rate threshold for the appearance of PVT was 110±10 bpm. Figure 3 shows a resting ECG and exercise-induced PVT in a patient from family 7.
All patients are treated with β-blockers (propranolol, 120±40 mg/d). A 22-year-old female patient is treated with 300 mg/d but continues to suffer from episodes of syncope. Eleven patients reported a complete resolution of symptoms, and in 2 patients with questionable treatment compliance (one of whom is mentioned above), the episodes of syncope continued although their frequency decreased. Since the initiation of this study (20 months), no deaths have occurred. Of the 9 children who died in these families, 5 were boys and 4 were girls. The average age at the onset of symptoms was 5±2 years, and average age at death was 7±4 years. None of those who died had been treated.

The parents of the affected individuals and other siblings (n=28) did not report any symptoms, had a normal physical examination, had a normal ECG and echocardiogram, and had no PVT on exercise test or isoproterenol infusion (data not shown).

**Table 1. Clinical Data on Patients With Catecholamine-Induced PVT**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at First Syncope, y</th>
<th>Age at Diagnosis, y</th>
<th>Current Age, y</th>
<th>Resting Heart Rate, bpm</th>
<th>QTc, s</th>
<th>VPB/PVT Threshold, bpm</th>
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<td>90</td>
<td>0.38</td>
<td>120</td>
<td>Syncope</td>
</tr>
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</table>

Mean±SD 6±3 9±8 13±7 64±13 0.4±0.02 110±10

VPB indicates ventricular premature beat.
*Bazzett’s formula.

**Linkage to Chromosome 1**

After testing the genome with nearly 300 microsatellites, linkage was initially detected with the marker D1S189. Subsequently, 6 additional chromosome-1 markers showed lod scores >3. Pairwise lod scores between the disease and chromosome-1 markers are presented in Table 2. A maximal 2-point lod score of 8.24 was obtained with the marker D1S189, at θ=0.00. Figure 1 shows typing results for the 7 families and 9 chromosome-1 markers. Individual 4-07 is recombinant for the marker D1S2849, defining the telomeric boundary of the interval containing the gene. Loss of homozygosity for the markers D1S2849 and D1S2868 in the 2 affected siblings of family 3 (first-cousin marriage) probably reflects a recombination event that occurred in one of the grandparents or great-grandparents and provides indirect support for D1S2868 as the telomeric boundary. Individual 7-04 is recombinant for the markers D1S534 and D1S514, defining D1S534 as the centromeric boundary. For 3 adjacent markers, D1S418, D1S189 and D1S2784, the same carrier allele (alleles 1, 1, and 1 respectively) could be observed in all carrier chromosomes. This haplotype was not found in any of the noncarrier chromosomes. The 2 markers located telomERICALLY to these 3 markers (D1S187 and D1S3723) show different carrier alleles in families 1 and 2 compared with families 3, 4, 5, 6, and 7. Families 1 and 2, although belonging to the same tribe, live in a community located several miles from the other families and have been separated from the other families for >50 years. The different carrier alleles observed for the markers D1S3723 and D1S187 probably represent historical recombination events, thus narrowing the disease interval to 16 megabases (Mb) between the markers D1S187 and D1S534.

**Exclusion of the KCND3 Gene**

KCND3 is located at the edge of the 16-Mb interval close to D1S187. This gene, which is highly expressed in cardiac tissue, is involved in I_o potassium current and in phase-1 cardiac action potential and was therefore regarded as a candidate gene. Sequencing of the entire coding region did not reveal any significant changes in the patients.

**Discussion**

In this report, we describe an autosomal recessive form of catecholamine-induced PVT and map the disease gene to the short arm of chromosome 1. Recombinant analysis placed the gene between D1S2849 and D1S534, a region spanning 48 Mb. Although 6 markers from within this interval did not show any recombinants, only 3 adjacent markers (D1S418, D1S189, and D1S2784) disclosed a common allele in all carrier chromosomes. This common haplotype is probably a remnant of the original founder chromosome, and the 2 different alleles observed at D1S187 result from recombinations that occurred in past generations. Thus, the use of
historical recombinants allowed us to further narrow the region to a 16-Mb interval between D1S187 and D1S534. Comparison of the families described in this report with those described by Leenhardt et al. show striking phenotypic similarity, especially in the age of onset and the resting heart rate. Lack of family history in two thirds of Leenhardt’s patients may suggest a recessive inheritance, increasing the likelihood that at least some of Leenhardt’s patients and the

Figure 3. A, Resting ECG of patient 7-04. B, Exercise-induced PVT in patient 7-04.

<table>
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<th>( Z_{\text{max}} )</th>
<th>( \theta_{\text{max}} )</th>
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\( Z_{\text{max}} \) indicates maximum lod score; \( \theta_{\text{max}} \) recombination fraction at \( Z_{\text{max}} \); and −∞, infinity.
Bedouin patients described here harbor mutations in a common gene. In contrast, the patients described by Swan et al differ from the Bedouin patients in an older age of onset, a lower penetrance, an older age at death, and a dominant mode of inheritance. It is therefore not surprising that the 2 disorders map to different loci.

The mean QT interval of the patients described in the present study, although in the normal range, was significantly longer than that of the unaffected siblings. The significance of this finding is unclear, but it may reflect a mild repolarization disorder as the underlying pathology.

Treatment with β-blockers was found to be effective in most of the patients. However, 2 patients, 19 and 22 years old, continued to suffer from recurrent syncope, and one of these had a documented episode of PVT. Poor treatment compliance seems to be the reason for failure in at least one of them, but inefficacy of β-blocker treatment due to insufficient dose or inherent incomplete efficacy cannot be ruled out. In such cases, the use of implantable defibrillators may be considered.

The 16-Mb interval between D1S187 and D1S334 contains 23 known genes and 53 known expressed sequence tags (ESTs). KCNJ3 encodes a potassium channel located on the edge of the interval near D1S187 and is highly expressed in cardiac tissue. Sudden death has been associated with a number of genes encoding cardiac sodium and potassium channel transporters. Therefore, KCNJ3 became the obvious candidate. However, no significant sequence alterations were found in affected individuals throughout the open reading frame of this gene. We have identified 3 additional interesting candidate genes from within this interval, all expressed in human heart tissue. NTRK1 encodes a cell surface receptor with strong homology to tyrosine kinase domain of growth factor receptors, though its function remains unknown. ATP1A1 encodes an integral membrane protein involved in electrochemical gradients of sodium and potassium ions across the plasma membrane. Sequence variants in this gene have been associated with salt-sensitive hypertension in rats, but theoretically mutations in the human gene could cause rhythm disturbances, especially in light of the crucial involvement of ion transporters in sudden death syndromes. ADORA3 encodes an adenosine receptor that, through its interaction with G proteins, inhibits adenylyl cyclase activity. Adenosine released during cardiac ischemia exerts a potent protective effect in the heart, and therefore, a defect in the receptor mediating this process could influence cardiac function under stressful conditions. Of the 53 ESTs confined to this region, 11 show expression in cardiac tissue and 1 is exclusive to cardiac cDNA libraries. Sequencing of these candidate genes and other cardiac-expressed genes and ESTs will eventually identify the gene causing this disease.

It is important to realize that the spectrum of catecholamine-induced PVT may extend beyond the small number of patients described to date in the literature. It may also include sporadic unexplained cases of exercise- or stress-induced sudden death in children, adolescents, and adults, as well as some cases of infant sudden death syndrome.

Acknowledgments

This study (No. 385/00-1) was supported by the Israel Science Foundation. We would like to thank Elaine Finkelstein and Dr Dirk Isbrandt for their assistance.

References

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_Circulation_. 2001;103:2822-2827
doi: 10.1161/01.CIR.103.23.2822

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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