Localization of a Gene Responsible for Arrhythmogenic Right Ventricular Dysplasia to Chromosome 3p23

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Background—Arrhythmogenic right ventricular dysplasia (ARVD), a familial cardiomyopathy occurring with a prevalence of 1 in 5000, is characterized by replacement of myocardium with fatty and fibrous tissue. Clinical manifestations include structural and functional abnormalities of the right ventricle and arrhythmias, leading to a sudden death rate of 2.5% per year. Four loci have been mapped, but no gene has been identified as yet.

Methods and Results—We identified a large family of ≥200 members with ARVD segregating as an autosomal dominant trait affecting 10 living individuals. The diagnosis of ARVD was based on international diagnostic criteria including history, physical examination, ECG, echocardiogram, right ventricular angiogram, endomyocardial biopsy, and 24-hour ambulatory ECG. Blood was collected for DNA from 149 family members. Analysis of 257 polymorphic microsatellite markers by genetic linkage excluded previously known loci for ARVD and identified a novel locus at 3p23. Analysis of an additional 20 markers further defined the region. A peak logarithm of the odds score of 6.91 was obtained with marker D3S3613 at θ=0% recombination. Haplotype analysis identified a shared region between markers D3S3610 and D3S3659 of 9.3 cM.

Conclusions—A novel locus for ARVD has been mapped to 3p23 and the region narrowed to 9.3 cM. Identification of the gene will allow genetic screening and a specific diagnosis for a disease with protean nonspecific findings. It should also provide insight fundamental to understanding cardiac chamber–specific gene expression and/or the mechanism of myocyte apoptosis observed in this disease. (Circulation. 1998;98:2791-2795.)

Key Words: apoptosis ■ cardiomyopathy ■ death, sudden ■ genetics ■ polymerase chain reaction

Arrhythmogenic right ventricular dysplasia (ARVD) is a familial cardiomyopathy of unknown cause characterized by replacement of myocardium with fatty and fibrous tissue. Its prevalence is estimated to be 1 in 5000,1 but it has been underrecognized because of the difficulty in diagnosing the disease. The primary lesion of ARVD is in the right ventricle, in contrast to the marked impairment of left ventricular function present in dilated cardiomyopathy. Methods for in vivo detection of functional or structural changes in the right ventricle remain insensitive and inadequate.1 Furthermore, the phenotype, other than sudden death, is so diffuse and nonspecific that an international task force developed diagnostic criteria based on arbitrary grading of an extensive list of clinical findings.2 Identification of the responsible gene(s) will be crucial for future genetic screening and will significantly improve diagnostic accuracy for this disorder. Despite the diagnostic difficulties,3 ARVD is now established as a major cause of sudden death in the young, occurring at a rate of 2.5% per year, frequently without prior symptoms. In Italy, it accounts for 20% of all sudden deaths in individuals <35 years old and 22% of sudden deaths in athletes.1

There are intriguing aspects of this disease with broad biological and pathological implications. First, the predominant involvement of the right ventricle, often with complete absence of involvement of the left ventricle, suggests chamber-specific expression of the defective gene. Second, the lesion shows evidence of apoptosis.1,4 Regardless of whether apoptosis is a primary or secondary process, this is a subject of active research in heart failure.5 Although no gene has yet been identified, 4 loci have been mapped (14q23,1 1q42,1 14q12,6 and 2q327) in families demonstrating autosomal dominant transmission. In addition, a form of the disease (Naxos disease) is coinherit with a skin disorder as an
autosomal recessive trait and maps to 17q21. We identified a large family of >200 members with 10 living affected individuals.

**Methods**

**Clinical Evaluation**
Informed consent was obtained according to the guidelines of Baylor College of Medicine and the Methodist Hospital. Individuals were evaluated by history, physical examination, and ECG. In addition, the following investigations were performed as appropriate: echocardiogram, right ventricular angiogram, endomyocardial biopsy, and 24-hour ambulatory ECG. A positive diagnosis for ARVD was made on the basis of the criteria proposed by the European Society of Cardiology and the International Society and Federation of Cardiology (ESC/ISFC). Individuals were classified as affected, normal, or indeterminate.

**Preparation of DNA**
Blood was collected from each family member. DNA was extracted, and cell lines were developed as previously reported.

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**Figure 1.** A, A 7-generation pedigree shows ARVD segregating as an autosomal dominant disease. This pedigree has been truncated to include only individuals used for linkage analysis and their immediate relatives. Solid symbols indicate affected members, those with a slash indicate deceased members, and those with a dot represent obligate carriers. B, Most likely haplotypes of selected individuals, including all 10 living affected individuals, 7 others (6 normal and 1 indeterminate) who appear to carry the disease-associated haplotype, 2 deceased affected whose genotypes could be inferred from offspring, and the offspring used to make this inference. Pedigree numbers correspond to the larger pedigree shown in A. Obligate carriers are indicated by symbols with a dot in center. Alleles surrounded by box represent alleles shared by all affected individuals.
Genotype Analysis

A total of 149 individuals were examined, and a pedigree was constructed. After diagnosis was complete, we truncated the pedigree to remove individuals who would not be useful for linkage analysis. The working pedigree is shown in Figure 1A. Individuals (living or deceased) who had affected individuals among their descendants but who were unavailable for analysis were designated as obligate carriers. We analyzed 56 individuals, including all living individuals shown in Figure 1A except for the following, who were not available: III:11, III:4, IV:13, IV:15, IV:17, IV:29, IV:31, V:10, V:12, V:14, V:22, V:34, V:37, V:41, VI:1, and VI:3. A genome scan was conducted using the linkage mapping set from Applied Biosystems. The set consists of 359 polymorphic microsatellite repeat markers located 10 to 15 cM apart. For each microsatellite marker, fluorescence-labeled primers were used to amplify fragments by polymerase chain reaction, and fragments were analyzed by capillary electrophoresis using an ABI model 310 genetic analyzer. Genomic regions exhibiting positive logarithm of the odds (LOD) scores were further explored by typing additional markers, primarily from the Genethon or NIH-CEPH genetic maps, as previously described.9

Linkage Analysis

Two-point linkage analysis was conducted on a personal computer using version 5.2 of the LINKAGE program.10 Multipoint linkage analysis was conducted on a VAX computer using FASTLINK. Autosomal dominant inheritance was assumed, and penetrance was set at 20% under age 15 years, 60% from age 15 to 35 years, 80% from age 35 to 55 years, and 95% over age 55 years, based on the observed frequency of affected individuals in at-risk sibships in this family (Figure 1A). Average overall penetrance in the family was 67%.

The allele frequencies for the disease and the normal alleles were assumed to be 0.0001 and 0.9999, respectively, and allele frequencies for microsatellite markers were arbitrarily set equal to 1/n, where "n" refers to the number of alleles observed.

Results

Clinical Findings

The family consists of >200 members spanning 7 generations (Figure 1). Data analysis of 149 individuals showed 10 affected and 1 indeterminate (IV:25), the latter having marginally decreased left ventricular ejection fraction. The remainder of the individuals were classified as normal. The diagnostic category for each individual was based on the criteria established by the ESC/ISFC,2 which proposed that 2 major, or 1 major and 2 minor, or 4 minor features be present. By definition, all affected individuals satisfied the major criterion of a positive family history. The diagnosis was confirmed in 5 individuals by histological analysis of right
ventricular biopsies (V:24, VI:4, VI:7, VI:19, VI:21) (Figure 1B). These individuals also exhibited other manifestations of ARVD, such as right ventricular dilatation and hypokinesis (V:24, VI:4), ventricular tachycardia (left bundle-branch block [LBBB] pattern) (V:24, VI:19), frequent ventricular extrasystoles with LBBB pattern (V:24, VI:7, VI:19, VI:21), and abnormal signal-averaged ECGs (V:24). The remaining affected individuals all had nonspecific depolarization, conduction, or repolarization abnormalities on ECGs (IV:18, IV:32, V:2, V:7, V:27) that, when coupled with other features, led to a positive diagnosis for ARVD. Ventricular tachycardia (LBBB pattern) was observed in IV:32 and frequent ventricular extrasystoles with LBBB pattern in V:27. Three individuals (IV:18, V:2, V:7) had ≥1 offspring who were confirmed to have ARVD on biopsy or on autopsy.

The pedigree indicates autosomal dominant inheritance. There were 17 individuals who died suddenly, ranging in age from 23 to 47 years (mean, 34±7 years), with 4 confirmed on autopsy to have ARVD. The 10 living affected individuals, half of whom are male, averaged 43 years of age at the time of diagnosis.

Linkage Analysis

We performed a random genome search on 56 individuals, including the 10 living affected, and identified the locus after analyzing 257 markers. An additional 20 markers were analyzed to further define the region of zero recombination. Two-point and multipoint linkage analyses were conducted. Among the regions excluded were the published loci for ARVD, including 14q23, 1q42, 14q12, and 2q32. Significant positive LOD scores (>3.0) were obtained with 5 markers (Table). A peak 2-point LOD score of 6.91 was obtained with marker D3S3613 at a recombination fraction of θ=0%, with 15 alleles segregating. No positive LOD scores >1.5 were seen for any other markers in the genome scan. Setting marker allele frequencies to those calculated from unrelated individuals did not markedly alter LOD scores. LOD scores were also robust for penetrance for the most closely linked markers and did not drop below 3 when penetrance was varied between 60% and 95% or when “affected only” analysis was performed. Haplotype analysis on the 10 living affected individuals and 2 deceased affected individuals whose genotype could be inferred from offspring identified a shared region flanked by markers D3S3610 and D3S3659 of 9.3 cM (Figure 1B). Genetic distances (in cM) between markers were determined from the Genethon map as D3S1263-(0.5)-D3S1259-(0.5)-D3S3610-(1.6)-D3S1585-(2.7)-D3S3613-(0.1)-D3S3473-(0)-D3S3595-(0.5)-D3S2338-(0.6)-D3S1293-(3.8)-D3S3659-(0.2)-D3S3700-(5.0)-D3S1266. The marker D3S1255 is not on the Genethon map and has been placed within the disease-associated haplotype on the basis of cosegregation in this family. Multipoint linkage analysis did not provide additional information concerning localization of the gene.

Discussion

Genetic linkage analysis of a large kindred indicates that a gene responsible for ARVD is located on the short arm of chromosome 3 (3p23). Uniform objective criteria were strictly followed in defining the disease. One individual who did not fully satisfy these criteria was classified as indeterminate. Families with ARVD can now be screened rapidly for linkage to those markers in the 3p23 region. It is of note that a locus for familial dilated cardiomyopathy has been mapped to this region. While the phenotype of our family is clearly that of ARVD, it is possible, although unlikely, that different defects in the same gene may give rise to both diseases. Possible candidate genes for ARVD in the 3p23 region include a raf serine-threonine protein kinase, a DNA-binding protein, and a protein-tyrosine phosphatase.

It is intriguing that the defect is usually in the right ventricle. In contrast, the left ventricle is primarily or exclusively involved in familial dilated and hypertrophic cardiomyopathies. Because the right ventricle is a low-pressure, low-energy system, it is not obvious which stimuli or conditions predispose the right over the left ventricle to ARVD. It is possible that the defective gene is preferentially expressed in the right ventricle, like dHAND. It will be exciting to determine whether the right ventricle is affected because of a chamber-specific stimulus, differential gene expression, or a combination of these. From a clinical viewpoint, identification of the gene will make genetic screening possible and will greatly improve our ability to diagnose the disease.

Acknowledgments

This work was supported in part by grants from the Medical Research Council of Canada Clinician Scientist Award, Phase I (Dr
Ahmad); the National Heart, Lung, and Blood Institute, Specialized Centers of Research (P50-HL54313-01-01), the National Institutes of Health Training Center in Molecular Cardiology (T32-HL07706), and the American Heart Association, Bugher Foundation Center for Molecular Biology (86–2216). We thank Drs Lawrence Sterns, Michael Kennedy, and George Klein for referring patients to us and for performing some of the clinical evaluations. We greatly appreciate the secretarial assistance of Debora Weaver and Valorie Garza in the preparation of the manuscript and figures.

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_Circulation_. 1998;98:2791-2795
doi: 10.1161/01.CIR.98.25.2791

_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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/98/25/2791

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