Gene for Arrhythmogenic Right Ventricular Cardiomyopathy With Diffuse Nonepidermolytic Palmoplantar Keratoderma and Woolly Hair (Naxos Disease) Maps to 17q21

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Background—Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heart muscle disease of unknown etiology that causes arrhythmias, heart failure, and sudden death. Diagnosis can be difficult, and this hampers investigation of its molecular basis. Forms of ARVC in which gene penetrance and disease expression are greater should facilitate genetic study. We undertook a clinical and genetic investigation of Naxos disease, originally described by Protonotarios in 1986. This disease constitutes the triad of ARVC, diffuse nonepidermolytic palmoplantar keratoderma, and woolly hair.

Methods and Results—We evaluated the population of Naxos, Greece, to identify probands, which was followed by family screening. Twenty-one affected persons from 9 families of 150 persons were identified. Linkage analysis was performed with microsatellite markers. The disease locus mapped to 17q21. A peak 2-point LOD score of 3.62 at \( \theta = 0.0 \) was found with a marker within intron 4 of the keratin 9 gene, a member of the type I (acidic) keratin family. A preserved homozygous disease haplotype was identified. Haplotype analysis delimited the disease interval.

Conclusions—Hair and skin abnormalities were found to be reliable markers of subsequent heart disease. This suggests the presence of a single mutant gene with novel cardiac, skin, and hair function or two or more tightly linked disease genes. Recessive inheritance of Naxos disease and a founder effect were demonstrated. Identification of a fully informative genetic marker linked to the disease and uncommon in the background population may be of use as a test to identify disease gene carriers. (Circulation. 1998;97:2049-2058.)

Key Words: genes ■ cardiomyopathy ■ Naxos disease

Arrhythmogenic right ventricular cardiomyopathy is a heart muscle disease pathologically characterized by the fibrofatty replacement of myocytes and extracellular matrix.1-3 The right ventricle is most frequently involved, but left-sided cardiac disease also occurs.4 The definitive diagnosis of ARVC requires the demonstration of fibrofatty replacement of the myocardium, which in life is usually not possible and furthermore may represent only advanced disease. Therefore, diagnostic criteria have been proposed by a combined ESC/ISFC task force.5

In ARVC, the main clinical problems are arrhythmia, heart failure, and sudden death.6 Risk factors for sudden death are relatively poorly delineated. However, ARVC appears to be relatively common in young persons, and particularly so in athletes. In athletes who died suddenly, between 3% and \( \approx 30\% \) were reported to have had ARVC.7-9 The disease may have both a variable presentation and a variable clinical course, even within a single family,10 and in many cases appears to be progressive.11,12 There is also a higher than expected rate of structural, functional, and ECG abnormalities in family members of persons with ARVC, and this may represent early disease.11,13-16 This clinical variation can make accurate diagnosis difficult, and because the correct determination of disease status is vital to accurate gene mapping and to subsequent gene identification, such diagnostic uncertainty may be significantly confounding.

Epidemiological data are limited, but there is evidence to support geographical clustering, for example in northern Italy,9 and this may suggest areas of high prevalence. In addition to sporadic cases, familial disease has frequently
been observed. In such families, mendelian inheritance has been reported. Usually the pattern of inheritance has been autosomal dominant, with various degrees of clinical expression. Observation of familial disease led to the hypothesis that a genetic abnormality may underlie cases of ARVC, and recently three gene loci were reported in families with autosomal dominant forms of the disease: 14q23–24 (ARVD1), 1q42 (ARVD2), and 14q12–22 (ARVD3). As yet, no disease-causing genes have been identified.

While investigating people affected by ARVC, we became particularly interested in a variant form recently named Naxos disease. This disease is characterized by typical cardiac features of ARVC, which are accompanied by diffuse NEPPK and WH, and has been reported only in people from the Greek island of Naxos. We hypothesized that the disease had a genetic basis and might have been introduced to the island from a single common ancestor. If so, this disease potentially represented a powerful model that could be of value to the determination of a molecular basis for ARVC, and therefore we examined the question, Is Naxos disease genetically determined? Further characterization of the molecular basis of Naxos disease may suggest a paradigm that could be useful to the determination of other forms of ARVC.

The PPKs are a genetically heterogeneous group of diseases. Diffuse NEPPK, similar to that seen in Naxos disease, is due to mutations in the type II keratin, keratin 1 (MIM *139350.4), which maps to 12q11–13. In contrast, focal NEPPK, dissimilar to that seen in Naxos disease, is due to mutations in the type I keratin, keratin 16 mapping to 17q21 (MIM *148700). Striated NEPPK (MIM 148700) maps to 18q12 in proximity to a desmosomal gene group, although as yet the specific mutation is unknown. WH (MIM *194300) is tightly curled hair occurring in non-Africans and is similar to that commonly seen in persons of African origin, in whom it is a normal trait. It has been observed as an isolated feature and as part of a disease syndrome. Autosomal dominant with male-to-male transmission, recessive, and sporadic forms have been described in non-African pedigrees. The molecular basis of WH is unknown.

In this study, we report our investigation of the molecular genetics of Naxos disease.

**Methods**

**Subjects**
The island of Naxos has ~20,000 inhabitants made up of ~200 apparently unrelated families, and we successfully screened all the families to recruit subjects for this study. This was possible because the only cardiologist on the island (N.P.) is a native of Naxos and knows all these families personally. To identify index cases, screening was particularly extensive in families with a history of cardiac, skin, or hair abnormalities. After identification of an index case, ascertainment was extended by use of public, medical, and church records, and all available family members were invited for study. Nine affected families were identified, from which 150 subjects were recruited for this study. Fifty apparently unrelated control subjects were randomly selected from 50 different unaffected Naxos families. All study and control subjects participated after informed consent had been obtained, and all subjects have been offered follow-up and counseling.

**Clinical Evaluation**
Noninvasive cardiac assessment was performed on all subjects. This included full clinical history and examination, resting 12-lead ECG, ambulatory 24-hour ECG, and two-dimensional transthoracic echocardiography. The cardiac diagnosis was based on ESC/ISFC guidelines. For the purposes of this investigation, no person underwent invasive evaluation beyond venous blood sampling. If clinical management reasons so indicated, coronary angiography, electrophysiological study, and endomyocardial biopsy were performed. The cardiac diagnosis was confirmed by pathological examination of tissue after surgery or endomyocardial biopsy in a subset of patients (n=3). Skin biopsy was performed in a subset of patients (n=3). Control parameters were established by noninvasive assessment of a group of 50 unrelated, healthy Naxians.

**Genetic Evaluation**
Pedigree analysis suggested autosomal recessive inheritance. Developing the earlier hypothesis of a founder effect, we hypothesized that a common ancestor had introduced a single copy of the disease gene to the genetically isolated population of Naxos, and then as a result of inbreeding, homozygous individuals arose with the phenotype of Naxos disease. This allowed the efficient and statistically powerful strategy of homozygosity mapping to be used to examine loci based on a candidate gene approach.

Thirty-eight of 150 family members and all 50 control subjects were available for additional molecular genetic analysis. Of the 21 affected people originally identified, there were 18 survivors (1 more died subsequently). Fourteen of them, along with 24 of their unaffected relatives, were available, including at least 1 affected person from each of the 9 families. In 3 of the 9 families, only the proband was available. DNA was extracted from peripheral blood leukocytes by standard methods. Subjects were genotyped by use of microsatellite markers by polymerase chain reaction, followed by electrophoresis in denaturing polyacrylamide gels and visualization by autoradiography, or by the LICOR DNA sequencer 4000L system. Polymerase chain reaction amplification was performed in a γ-32P or infrared dye-labeled reaction with 30 cycles at 94°C for 30 seconds, annealing for 60 seconds, and extension at 72°C for 60 seconds. Oligonucleotides were supplied from Research Genetics or derived from markers linked to the candidate genes. D17S1294, D17S1293, D17S800, D17S1299, and D17S809 are positioned according to data from markers linked to the candidate genes. D17S1294, D17S1293, Oligonucleotides were supplied from Research Genetics or derived from markers linked to the candidate genes. D17S1294, D17S1293, D17S800, D17S1299, and D17S809 are positioned according to data from the Location Database (LDB). Genome Database (GDB), and other high-resolution map data of this region. Linkage analysis was performed with the C version of the LINKAGE package, FASTLINK, and the utility programs Makeped, LCP, LRP, and UNKNOWN, from LINKAGE 5.1. Population allele frequencies were derived from the 50 Naxian control subjects and not from published data from other populations that may not have been representative.

**Results**

**Clinical Features**
In every affected person, all three components of the Naxos disease phenotype were observed together. There were no...
cases in which only a solitary feature or two of the three features occurred. In all affected persons, WH was present from birth and persisted throughout life (Fig 1A). Palmoplantar erythema was present at or developed shortly after birth and progressed to a diffuse NEPPK (Fig 1B, 1C, and 1D). The cardiac abnormality (Fig 2) was not clinically manifest until the patient was ≈15 years of age, and onset was usually with palpitation or syncope. Three patients died during follow-up: 2 suffered sudden death (Fig 3, marked as *SD), and the other developed progressive cardiac failure (Fig 3, marked as *HF). There were also 4 additional recently deceased persons who were known to have had WH and PPK. In 3 of the 4, data from their medical records supported a diagnosis of ARVC (Fig 3, marked as ND). In the other deceased person (Fig 3, marked as ?ND), no additional cardiac data were available.

Figure 1. A. WH as a feature of Naxos disease. Posterior view showing tight curly hair in person with Naxos disease (non-African ancestry). B and C, Palmar and plantar keratoderma in Naxos disease. Note clear demarcation at border with dorsal skin. D, Histopathological preparation of palmar skin biopsy. Severe hyperkeratosis without epidermolysis.
Pedigree Analysis

We identified 9 affected families (Fig 3). These families were derived from 11 apparently unrelated Naxian families. Family trees could be constructed for up to 7 generations back from the most recent, and no other relationships could be identified between the kindreds to this level. One hundred fifty living family members were recruited into the study, from whom 21 living affected persons were identified (9 female, 12 male; age, 7 to 74 years, with a mean of 38 years at first evaluation; age, 18 to 76 years, with a mean of 50 years at last review). Pedigree inspection suggested autosomal recessive inheritance of Naxos disease.

Linkage Analysis

Thirty-eight family members comprising 14 people with Naxos disease and 24 of their unaffected relatives were available for molecular genetic analysis. At least 1 affected person was available from each of the 9 families. In 3 of the 9 families, only the proband was available for molecular analysis. By linkage analysis, the following loci were excluded: the three loci for autosomal dominant ARVC19–21; two loci on chromosome 1 implicated in dilated cardiomyopathy39,40; also on chromosome 1, the epidermal differentiation complex41; the dystrophia myotonica protein kinase CTG repeat locus on 19q1342; the type II keratin cluster on 12q26,27; and the locus on 18q implicated in striated NEPPK.28 Two-point linkage analysis between markers D17S1294, D17S1293, D17S800, KRT9, THRA1, D17S1299, and D17S809 gave evidence for linkage of Naxos disease to 17q. The results of 2-point analyses for each of these markers analyzed are shown in Table 1. The peak 2-point LOD score was 3.62 at θ=0.0 with the fully informative marker KRT9,43 giving evidence for linkage of Naxos disease to 17q21. The KRT9 allele 1, which cosegregated exclusively with the disease, had an observed allele frequency of only 6.9% in the control group, and this frequency was used for linkage analysis. The frequency of this allele in the Naxos control population was similar to that previously reported in another control group (6%).43 The homozygous 11 genotype for KRT9 was seen only in affected persons and not in their healthy family members or in control subjects (Table 2 and Fig 3). Haplotype analysis localizes the Naxos disease gene to a region of ~7 cM on 17q21 flanked by the markers D17S800 and D17S80912,33 (Table 2). A haplotype unique to all the affected individuals is observed with 3 markers and comprises only homozygous genotypes. The disease haplotype is given by the markers KRT9-THRA1-D17S1299. Of the 14 affected persons, 3 (E11, I13, and K17) did not have other family members available for molecular analysis and therefore do not contribute to the LOD score, suggesting that this estimate of linkage is likely to be conservative.

Discussion

A challenge to the evaluation of people and families with ARVC is the accurate identification of disease status. This reflects the variable clinical expression of ARVC as well as the limitations inherent to current methods of cardiac assessment. Clinical evaluation of families from the island of Naxos, Greece, identified a syndrome in which WH and PPK are predictive of the development of ARVC.22 In contrast to...
many cases of ARVC, this represented a disease variant in which diagnostic status could be more confidently assigned. This model is made more robust because in an isolated community, both clinical and genetic heterogeneity are often reduced, making disease phenocopies less likely.44 The mode of inheritance appeared to be autosomal recessive. Linkage analysis mapped the disease locus to 17q21, strongly supporting a genetic basis for this disease. A homozygously inherited disease haplotype that is common to all the affected individuals was identified, which supports an autosomal recessive mode of inheritance as well as a founder effect. This haplotype delimits the region of the disease gene to \( \approx 7 \) cM. Identification of a fully informative molecular marker linked to the disease, infrequent in the background population, and not seen in a homozygous form in healthy relatives or control subjects could be used as a genetic test to identify risk status among Naxians. This investigation also identifies a locus for WH.

The exclusion of linkage to reported ARVC loci and the identification of a novel locus for Naxos disease implies a different, although still possibly related molecular basis between the diseases. Given the historical links with other Mediterranean peoples, it had been suggested that ARVC on Naxos and that seen in Italy had a common molecular basis. The previous mapping studies had all included families from Italy. In the study that identified the ARVD1 locus at 14q23–24, a large multigenerational Italian family had been investigated, and linkage to 17q21 had been excluded15; this represents additional support for a distinct molecular basis between this variant of ARVC and Naxos disease. Further evaluation of other populations and families affected by ARVC for linkage to 17q21 could be of value in two ways. First, if linkage is identified in a given family, such an observation may assist in assigning disease status to additional family members of clinically undetermined phenotype. Second, if other ARVC families do demonstrate linkage to 17q21, the region in which there is a common or overlapping conserved haplotype between the Naxos disease families and the ARVC families may be significantly narrower. This would benefit identification of an etiological gene by localizing it to a smaller interval.

To explain the broad features of the phenotype, it is necessary to consider two models: first, that the disorder may arise as a result of mutation in a single gene that plays a critical role in more than one biological pathway. Alternatively, mutation affecting two or more tightly linked genes may produce the disease phenotype. There is precedent for both of these models.45–47 This region of 17q21 is gene rich and has been well explored as a consequence of its proximity to \( \text{BRCA1} \) (breast cancer gene type 1). The disease haplotype is in proximity to a number of candidate genes. In addition to at least 10 genes of the type 1 keratin group,23 there are genes involved in morphogenesis48 and the maintenance of sar-
Figure 3. Pedigrees of families affected by Naxos disease. Haplotypes of family members available for molecular analysis reveal a conserved disease haplotype (KRT9-THRA1-D17S1299) inherited in autosomal recessive fashion, suggesting that these families are all ancestrally related and that Naxos disease is the consequence of a founder effect. *SD indicates sudden death during follow-up; *HF, progressive cardiac failure leading to death during follow-up; ND, 3 of the 4 persons who died suddenly reported to have PPK and WH with data from medical records supporting a diagnosis of ARVC; and ?ND, 1 of 4 persons who died suddenly reported to have PPK and WH and no additional medical data available.
Figure 3 Continued.
Keratins are part of the intermediate filament family and, like other members of this structurally and functionally related group, are known to have a role in cell stability. Eight mutations have been identified in keratin 9,43-46 All were exonic and involved the conserved coil 1A of the rod domain, important in heterodimerization and keratin stability. In all those cases, the clinical pattern was of an epidermolytic PPK, different from the diffuse NEPPK of Naxos disease. This does not exclude keratin 9, and mutation analysis of this gene will be an important part of the search for the etiological mutation. The keratin 9 intragenic marker linked to Naxos disease is a short sequence repeat of the form (TG)z, and lies within intron 4. The effect on gene function produced by this recessively inherited intronic sequence variation cannot be reliably predicted. Therefore, because of this and because keratin 9 is thought to be expressed in mature palmitoplantar epithermis but not in the heart,63,64 we are extending our evaluation. This will include assessment of neighboring genes as well as an investigation of intermediate filament gene expression in the heart.

Support for the paradigm of an intermediate filament abnormality per se, or an intermediate filament–related gene abnormality, stems from the recent identification of mutation in the gene for plectin as the cause of an autosomal recessive muscular dystrophy accompanied by epidermolysis bullosa simplex.45 Plectin is involved in intermediate filament attachment to the cell membrane in both epithelial and muscle cells. In skin, plectin deficiency leads to a failure of keratin filaments to attach to the plasma membrane via hemidesmosomes, whereas in muscle there is abnormal localization of the muscle intermediate filament desmin. The net result is cellular fragility producing skin and muscle disease.45 It is of interest that in this syndrome, as in Naxos disease, skin disease clinically precedes the muscular abnormalities.

In ancient times, Naxos was the predominant island of the Aegean, its wealth enabling it to export marble to mainland Greece for the construction of temples and to supply large numbers of troops. Later it saw a relative decline and in 1204 CE was annexed by Venice. For this and other complex cultural reasons, a strong tradition developed and remains for Naxians to have children only with other Naxians, although not with close relatives. The people of Naxos screen their children and know from their folklore that if they are born with the hair and skin stigmata, they may die young. Identification of linkage to 17q21 and a haplotype for Naxos disease offers a test to identify risk status for the people of Naxos. In this study, the genotype 11 for the KRT9 marker was observed only in clinically affected persons, and this implies that the KRT9 allele is in strong linkage disequilibrium with the disease gene or genes. In turn, this suggests that the heterozygous state 1/z (where z is any other allele) for the KRT9 marker identifies the carrier state among the Naxos population. With appropriate counseling and fully informed consent, it will be possible to advise Naxians of the relative risk to their offspring of Naxos disease.

Subsequent identification of the genetic abnormality in Naxos disease may suggest related genes as candidates for other forms of ARVC. This may also benefit our understanding of clinically overlapping syndromes, which include right bundle-branch block/persistent ST-segment elevation/sudden death syndrome,65 Uhl’s anomaly,66 and ARVC plus mitral valve prolapse syndrome,67 and diseases that have both cardiac and ectodermal abnormalities, such as Noonan syndrome,68 cardiofaciocutaneous syndrome,69 and LEOPARD syndrome.70 In broader terms, an understanding of the molecular basis of ARVC may benefit our understanding of the biology of myocyte regulation, arrhythmogenesis, and heart failure.

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