

IDENTIFICATION OF A GENETIC LOCUS FOR FAMILIAL ATRIAL FIBRILLATION

RAMON BRUGADA, M.D., TERRY TAPSCOTT, B.S., GRAZYNA Z. CZERNUSZEWICZ, M.S., A.J. MARIAN, M.D., ANNA IGLESIAS, B.S., LLUIS MONT, M.D., JOSEP BRUGADA, M.D., JOSEP GIRONA, M.D., ANNA DOMINGO, M.D., LINDA L. BACHINSKI, PH.D., AND ROBERT ROBERTS, M.D.

ABSTRACT

Background Atrial fibrillation, the most common sustained cardiac-rhythm disturbance, affects over 2 million Americans and accounts for one third of all strokes in patients over 65 years of age. The molecular basis for atrial fibrillation is unknown, and palliative therapy is used to control the ventricular rate and prevent systemic emboli. We identified a family of 26 members of whom 10 had atrial fibrillation that segregated as an autosomal dominant disease. We subsequently identified two additional families in which the disease was linked to the same locus.

Methods We screened the human genome with 300 polymorphic dinucleotide-repeat markers using an unconventional strategy of pooling the DNA samples into two groups (affected and unaffected), which reduced the sample size by approximately 90 percent, before performing linkage analysis to map the locus. This made it possible to identify potential loci within a few weeks.

Results The lod scores for markers D10S569 and D10S607, located at 10q22-q24, were 3.60 in Family 1. The disease locus in Families 2 and 3 was also linked to the same markers, with lod scores of 6.02 and 5.35 for markers D10S569 and D10S607, respectively, when data on all three families were combined. Haplotype analysis of the three families showed that the locus was between D10S1694 and D10S1786, an interval of 11.3 centimorgans.

Conclusions Identification of the gene for familial atrial fibrillation will help to elucidate the molecular basis of the disease and provide insights into acquired forms. The strategy of pooling DNA samples for analysis is more time and cost effective than conventional screening and should accelerate the process of gene mapping in the future. (N Engl J Med 1997;336:905-11.)

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ATRIAL fibrillation, the most common sustained cardiac-rhythm disturbance,¹ affects more than 2 million Americans,² with an overall prevalence of 0.89 percent. The prevalence increases rapidly with age to 2.3 percent between the ages of 40 and 60 years and to 5.9 percent over the age of 65.² Although the initial course of atrial fibrillation is often paroxysmal, it almost invariably progresses to a chronic sustained rhythm disturbance with manifestations ranging from palpitations to cardiac failure. The most dreaded com-

plication is stroke; atrial fibrillation accounts for one third of all strokes in patients over the age of 65.³ Since there is currently no effective means of preventing or eliminating atrial fibrillation, therapy consists of controlling the ventricular rate and preventing systemic emboli with antiplatelet and anticoagulant therapy.⁴ The emotional and medical burdens imposed by long-term medical therapy and, in many cases, subsequent stroke are immense, with a total annual cost of \$9 billion.

The molecular basis of atrial fibrillation has yet to be determined. One approach, which has been successful for metabolic disorders, is to identify the gene responsible for a familial form of the disease. Familial atrial fibrillation is probably very uncommon,⁵ but we located a small family in Spain in which atrial fibrillation segregates as an autosomal dominant trait. Only 10 living family members were affected; thus, to map the chromosomal locus by conventional genetic-linkage analysis would probably require analyzing DNA markers every 5 to 10 centimorgans (cM) throughout the human genome. The arduous task of analyzing DNA from each of the 26 family members for 300 to 600 markers would also be very costly. Accordingly, we adopted an unconventional strategy of pooling the DNA of the affected family members and comparing the results of the DNA analysis with those of an analysis of pooled DNA from unaffected family members at each marker locus to detect differences that would suggest segregation of a particular allele with the disease. This reduced the number of samples to be analyzed by more than 90 percent and enabled us to identify four potential loci within a few weeks.

METHODS**Study Families and Diagnosis**

We identified a family (Family 1) in which atrial fibrillation appears to be segregating as an autosomal dominant disease with high penetrance. The family consists of 26 living members spanning three generations, of whom 10 have atrial fibrillation. Two additional family members are known to have died of complica-

From the Department of Cardiology, Baylor College of Medicine, Houston (R.B., T.T., G.Z.C., A.J.M., A.I., L.L.B., R.R.); the Cardiac Arrhythmia Service, Department of Cardiology, Hospital Clinic, University of Barcelona, Barcelona, Spain (L.M., J.B.); and the Department of Cardiology, Hospital Materno-Infantil Vall d'Hebron, Barcelona, Spain (J.G., A.D.). Address reprint requests to Dr. Roberts at Baylor College of Medicine, 6550 Fannin, MS SM677, Houston, TX 77030.

tions of the disease. We subsequently identified two other small families with a total of 17 living members, 9 of whom are affected. In these two families (Families 2 and 3) the defect mapped to the same locus as in the original family. The three pedigrees are shown in Figure 1.

Clinical Evaluation

After providing informed consent according to the guidelines of Baylor College of Medicine and Methodist Hospital, the subjects were evaluated by a detailed history taking, physical examination, 12-lead electrocardiography, and two-dimensional echocardiography. Criteria for diagnosis were based on the electrocardiographic findings. Other likely causes of atrial fibrillation in this population — such as hypertension, valvular disease, and thyroid disease — were ruled out at the time of diagnosis.

Preparation of DNA and Pooled Samples

Blood was collected from each member of the three families, DNA was extracted by the salting-out procedure,⁶ and lymphocytes were isolated for the development of transformed cell lines.⁷ For the pooled-sample analysis we used blood only from Family 1. Equimolar amounts of DNA from 10 affected members (Subjects II-2, II-3, III-1, III-3, III-5, III-11, IV-6, IV-7, IV-8, and IV-9) were combined into a single sample, as was DNA from 10

unaffected family members (Subjects II-1, II-4, II-6, III-4, III-6, III-8, III-9, III-10, III-12, and IV-5). Whenever possible, unaffected siblings and parents of affected subjects were chosen to provide samples for the unaffected pool in order to minimize the allelic differences between the two groups.

Marker Analysis

A total of about 300 polymorphic dinucleotide — (CA)_n — repeat markers located approximately 15 to 20 cM apart were selected on the basis of their polymorphic information content primarily from the genetic maps of Genethon⁸ or the National Institutes of Health—Centre d’Etude du Polymorphisme Humain.⁹ For each microsatellite marker, primers annealing to the sense strand were end-labeled with [³²P]γ-deoxyadenosine triphosphate with polynucleotide kinase (Pharmacia), and the DNA was amplified by the polymerase chain reaction under standard conditions for 30 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. Some markers required a higher annealing temperature. Initial denaturation was carried out at 95°C for five minutes. Each 50-μl reaction contained 150 pmol of specific primers, 0.3 U of *Taq* polymerase (Pharmacia), 200 μM 4-deoxynucleoside triphosphate, and 200 ng of genomic DNA for the analysis of either individual or pooled DNA samples. The ampli-

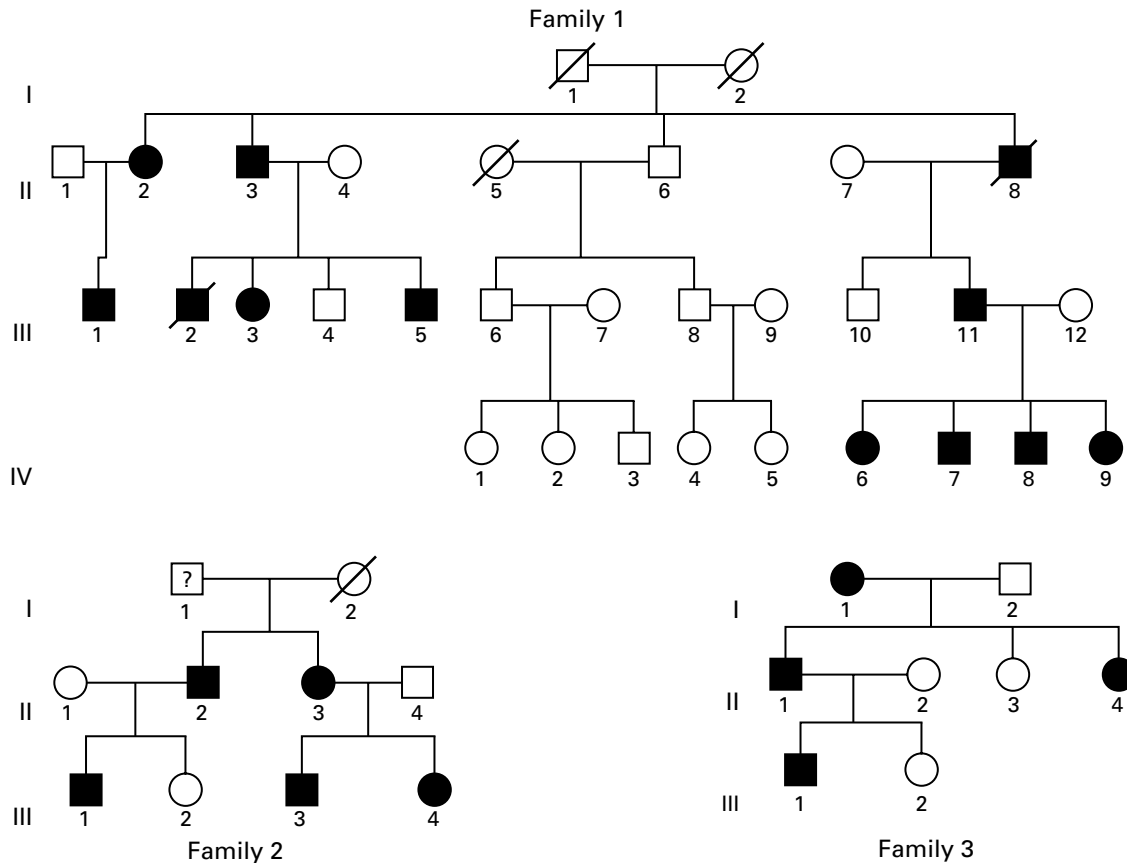


Figure 1. Pedigrees of Three Families with Familial Atrial Fibrillation.

Circles denote female family members, squares male family members, solid symbols affected family members, symbols with a slash deceased family members, and the symbol with a question mark a family member whose disease status has not been determined.

fied DNA products were analyzed by electrophoresis through 6 percent denaturing polyacrylamide-urea sequencing gels as previously described.¹⁰

After the 300 chromosomal markers were amplified from the two pooled DNA samples, the products were loaded side by side on a polyacrylamide gel and then subjected to electrophoresis. The electrophoretic pattern exhibited on the autoradiograph by each marker allele amplified from the pooled sample from the affected group was visually inspected, and the results were compared with those of the pooled sample from the unaffected group. The presence of a unique band or a band of greater intensity in the sample from the affected group suggested cosegregation of the marker allele with the disease allele.

Linkage Analysis

Two-point linkage analysis was carried out on a personal computer with version 5.2 of the Linkage program.¹¹ Multipoint linkage analysis was conducted on a Vax computer with Fastlink. An autosomal dominant pattern of inheritance was assumed, and penetrance was set at 99 percent, on the basis of the observed high frequency of affected persons in sibships at risk in Family 1. The frequencies of the disease allele and the normal allele were assumed to be 0.0001 and 0.9999, respectively, and the allele frequencies of microsatellite markers were arbitrarily assigned a value of $1/n$, where n refers to the number of alleles observed. Since the use of incorrect values for linkage parameters can cause false positive results, positive lod scores were tested for robustness with respect to variations in penetrance (from 60 to 99 percent), variations in the prevalence of phenocopies (from 0 to 5 percent), and marker-allele frequency with the use of published frequencies, when available, or the frequencies observed in this family.

RESULTS

Clinical Characteristics

In Family 1 there were 10 living affected subjects (6 male and 4 female) whose age at diagnosis ranged from 2 to 35 years (average, 17.8). Nine family members had chronic atrial fibrillation, and one (Subject IV-6) had paroxysmal atrial fibrillation. Three had dyspnea on exertion (Subjects IV-6, II-2, and III-5), and seven were asymptomatic. Subject III-1 had pericarditis and atrial fibrillation at the time of diagnosis, but since the atrial fibrillation at the time of diagnosis, but since the atrial fibrillation has persisted for 10 years, pericarditis was not considered the cause. Subject II-8 died of a cerebrovascular accident at the age of 68. Subject III-2, who was given a diagnosis of paroxysmal atrial fibrillation at the age of 20, died suddenly at the age of 36; no autopsy was performed. Electrocardioversion was attempted unsuccessfully in three of those with chronic atrial fibrillation. Subjects II-2 and III-5 had increased left ventricular internal diameters and ventricular ejection fractions of 51 and 54 percent, respectively. The remainder had no abnormalities on echocardiography, and the average ejection fraction was 69 percent.

In Families 2 and 3, all nine affected subjects had chronic atrial fibrillation, were asymptomatic, and had no echocardiographic abnormalities. The age at diagnosis in these two families ranged from 2 to 46 years.

DNA Analysis and Genetic Linkage

The 300 chromosomal markers used to screen the human genome were amplified from the pooled DNA samples from affected and unaffected subjects; the pooling procedure reduced the number of samples to be analyzed from 26 to 2. The electrophoretic pattern of the sample from the affected group differed from that of the unaffected group for 50 of the 300 marker alleles. These differences, which suggested potential loci for the gene responsible for atrial fibrillation, were identified within a few weeks. A typical example of a difference in a marker allele between the pooled samples is shown in Figure 2. In lanes 1 and 2 and lanes 3 and 4 of Figure 2, the electrophoretic pattern is similar, indicating that the two marker alleles are segregating at random with respect to the disease allele. In contrast, the intensity of the uppermost allele band in lane 6, showing the sample from the affected group, is greater than the intensity of the corresponding allele in lane 5, showing the sample from the unaffected group. A marker allele with greater intensity in the affected group,

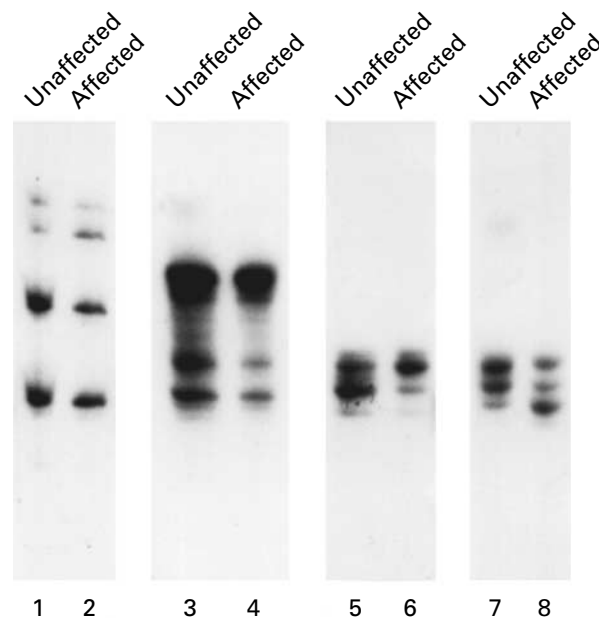


Figure 2. Results of Polyacrylamide-Gel Electrophoresis of Four DNA-Marker Loci Amplified from Pooled DNA.

In lanes 1 and 2 and lanes 3 and 4 the electrophoretic patterns are identical for the markers amplified from the pooled DNA samples from the unaffected and affected groups. The samples shown in lanes 5 and 6 and lanes 7 and 8 show typical differences in band intensity observed between the two groups. The intensity of the lowermost band in lane 8 is much greater than that of the corresponding band in lane 7, reflecting an increase in the number of copies of that allele in the affected group that is consistent with the occurrence of cosegregation of the marker allele and the disease allele.

TABLE 1. TWO-POINT LOD SCORES FOR FOUR INFORMATIVE MARKERS LINKED TO 10q22–q24.*

MARKER AND FAMILY NO.	FREQUENCY OF RECOMBINATION (θ)					
	0.00	0.01	0.05	0.1	0.2	0.3
	lod score					
D10S569						
3	1.20	1.17	1.08	0.97	0.72	0.46
2	1.14	1.11	1.01	0.88	0.61	0.34
1	3.69	3.63	3.38	3.05	2.34	1.54
Total	6.02	5.91	5.47	4.90	3.67	2.34
D10S607						
3	0.60	0.59	0.55	0.51	0.41	0.29
2	1.05	1.03	0.93	0.80	0.54	0.29
1	3.70	3.64	3.39	3.06	2.34	1.55
Total	5.35	5.25	4.87	4.37	3.29	2.13
D10S1677						
3	0.60	0.58	0.53	0.46	0.32	0.17
2	1.05	1.03	0.93	0.80	0.54	0.29
1	3.60	3.54	3.29	2.96	2.25	1.45
Total	5.25	5.16	4.75	4.23	3.11	1.91
D10S1689						
3	1.20	1.17	1.08	0.97	0.72	0.46
2	1.07	1.05	0.95	0.82	0.56	0.30
1	-3.03	0.38	1.04	1.22	1.12	0.79
Total	-0.76	2.60	3.08	3.01	2.40	1.55

*The maximal lod score for each marker is shown in boldface. The lod scores have been rounded.

presumably due to an increased number of copies of the allele, suggests cosegregation with the disease.

To confirm or rule out cosegregation, genetic-linkage analysis was necessary. This required genotyping of DNA from each of the 26 members of Family 1 for each of the 50 marker alleles. The resulting lod scores were at least -2 for all but four loci, for which low, positive scores were obtained that were consistent with the occurrence of cosegregation of the marker allele and the disease allele. Additional markers located in these four regions were amplified and analyzed, and the results ruled out three of these regions (4q, 7q, and 13q), but marker alleles (D10S569 and D10S607) in the region 10q22 cosegregated with the disease allele, with maximal two-point lod scores of 3.60, indicating genetic linkage. Lod scores for these markers remained above 3.0 despite variations in penetrance from 60 to 99 percent.

Since the estimated prevalence of atrial fibrillation in the general population below the age of 40 is less than 0.04 percent² and the nonfamilial causes of atrial fibrillation at this young age are almost exclusively related to valvular heart disease or congenital defects, a phenocopy prevalence of even 1 percent is probably excessive. Nevertheless, lod scores for these markers remained significant even with a phenocopy prevalence of 5 percent. Similarly, lod scores were not sensitive to variations in the estimated frequencies of marker alleles.

We also typed a number of markers in Families 2

and 3 and determined that they had the same haplotype on the affected chromosome as Family 1 for all markers from D10S188 to D10S219, suggesting that the three families are distantly related. Markers outside this interval (D10S581, D10S1694, D10S1786, and D10S1686) were associated with different alleles in the other two families. A total of 27 markers were examined in an effort to define the precise boundaries of the region, but not all these markers were informative. Lod scores for the four most informative markers are presented in Table 1. Multipoint analyses resulted in a peak lod score of 3.60 between markers D10S569 and D10S607 for Family 1 alone and 6.17 for all three families combined and contributed no new information with respect to the location of the gene for atrial fibrillation.

Figures 3, 4, and 5 show the haplotypes for 11 informative markers in all three families and the positions of the recombination events that helped determine the location of the gene. In Family 1 crossovers were observed between the disease allele and D10S1786 in Subject III-1 and between the disease allele and D10S581 in Subject IV-6. Thus, on the basis of haplotype analysis, the gene causing atrial fibrillation in Family 1 lies between D10S581 and D10S1786, an interval of approximately 20.2 cM, on chromosome 10q22–q24. Family 3 had no crossovers in this region. In Family 2, either Subject II-2 or Subject II-3 had a crossover between D10S1694 and the disease allele, further limiting the region to one measuring 11.3 cM. Affected members of all three families had identical alleles for all markers tested from D10S188 through D10S219. Because the haplotype was conserved throughout such a large region, we conclude that these three families must be distantly related and have the same mutation at this locus, supporting the conclusion that the disease gene lies proximal to D10S1786 and distal to D10S1694, an interval of 11.3 cM.

DISCUSSION

We identified a small family in whom atrial fibrillation segregated as an autosomal dominant trait; this family consisted of 26 members, 10 of whom were affected and alive at the time of the study. Genetic-linkage analysis indicated that the gene responsible for atrial fibrillation in this family is located on chromosome 10q in the region of 10q22–q24, with two markers having maximal lod scores of 3.60. We identified regions containing potential loci for this gene within a few weeks by comparing the electrophoretic patterns exhibited by markers amplified from pooled DNA samples from the affected group with those observed in the pooled DNA samples from the unaffected parents and siblings, rather than by genotyping each marker in all 26 family members. This procedure reduced the number of samples in the genomic screen by 90 percent. Individual DNA

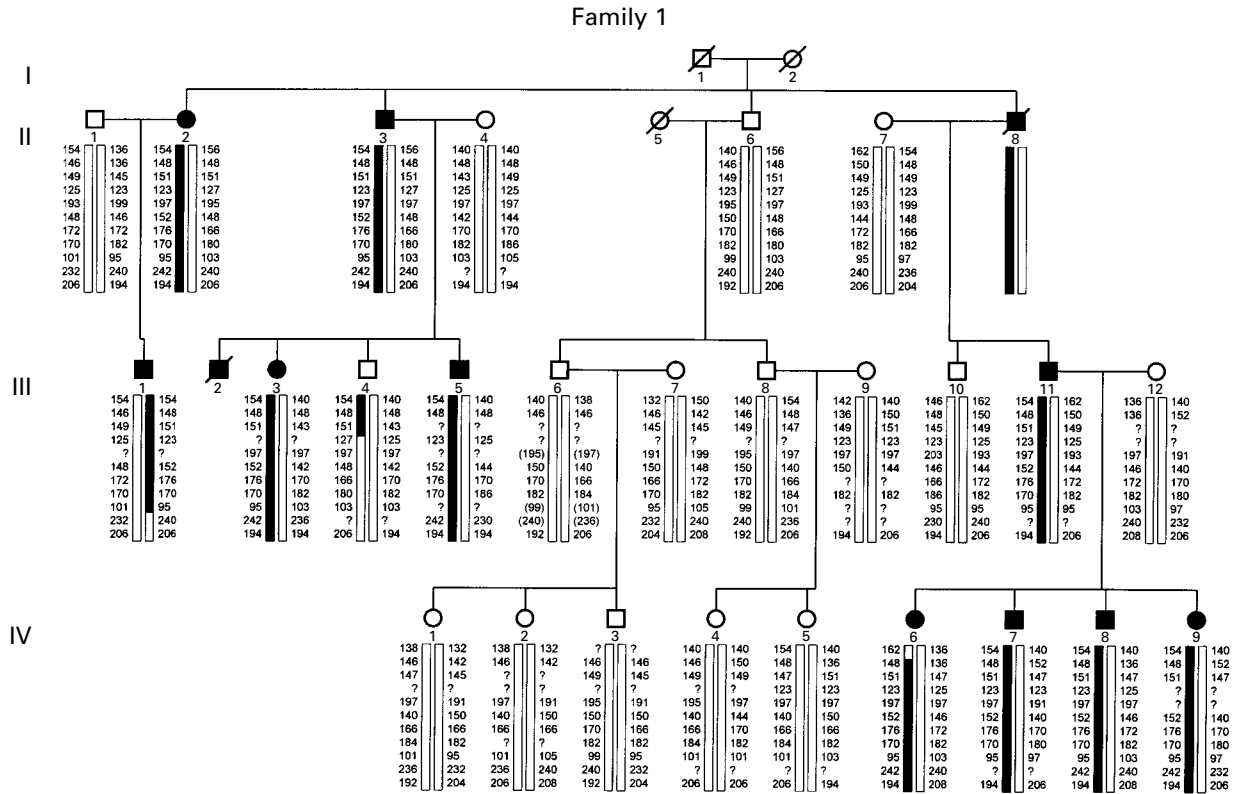


Figure 3. Haplotypes and Recombination in Family 1.

Alleles are shown as base-pair sizes in centromere-to-telomere order for markers D10S581, D10S537, D10S1694, D10S188, D10S556, D10S569, D10S607, D10S1677, D10S219, D10S1786, and D10S1686. Alleles in parentheses represent inferred genotypes. For genotypes represented by question marks, no data were available. The most highly conserved haplotypes are assumed, and the chromosomal region cosegregating with the disease locus in each family is represented by the black bar. No attempt has been made to show crossover on the nondiseased chromosome. Circles denote female family members, squares male family members, solid symbols affected family members, and symbols with a slash deceased family members.

analyses were performed for only 50 markers (a reduction of approximately 75 percent), resulting in the identification of the locus within a few weeks as opposed to several months to a year. The strategy gives rapid results and should be particularly useful in small families with a high penetrance of the disease gene. Subsequently, we identified two other families with atrial fibrillation that was also linked to 10q22–q24 (combined lod score for all three families, 6.02). The odds of genetic linkage of the disease in these families to the region of 10q22–q24 remained significant despite the wide range of values used for the variables in the linkage analyses — namely, a phenocopy prevalence of up to 5 percent and penetrance ranging from 60 to 99 percent. This analysis provides the first essential step in the identification of the responsible molecular defect.

The pooled-sample approach is based on the fact that each DNA marker has a unique and characteristic distribution of allele frequencies in the general population. When the DNA is pooled into two

groups (affected and unaffected), each sample contains many alleles (rather than two, as is the case for a single subject), and thus, the intensity of the electrophoretic bands is dependent on the frequencies with which those alleles are represented in the pool. If a marker allele is segregating at random with respect to a disease, its distribution in the affected and unaffected subjects will be identical. If, on the other hand, a particular marker allele is cosegregating with the disease allele, then all the members of the affected pool will share this allele and DNA analysis will show a band of greater intensity than in the sample from the unaffected subjects or a unique band. The strategy of using pooled DNA samples to look for differences in allele distribution has been used to identify the locus of a recessive disease in a highly inbred population.¹² Its lack of use in autosomal dominant diseases is probably due to the expectation that the inordinately high incidence of differences between affected and unaffected samples would represent false positive results. We minimized false posi-

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