Locus for Atrial Fibrillation Maps to Chromosome 6q14–16

Patrick T. Ellinor, MD, PhD; Jordan T. Shin, MD, PhD; Rachel K. Moore, BS; Danita M. Yoerger, MD; Calum A. MacRae, MB, ChB

Background—Atrial fibrillation (AF), the most common clinical arrhythmia, is a major cause of morbidity and mortality. Although AF is often associated with other cardiovascular conditions, many patients present without an obvious etiology. Inherited forms of AF exist, but the causative gene has been defined only in a single family. We have identified a large family (family FAF-1) in which AF segregates as a Mendelian trait.

Methods and Results—Thirty-four family members were evaluated by 12-lead ECG, echocardiogram, 24-hour Holter monitoring, and laboratory studies. Individuals with electrocardiographically documented AF were defined as affected. Subjects were considered unaffected if they were >60 years of age, had no personal history of AF, and had no offspring with a history of AF. DNA was extracted and genotypic analyses were performed using polymorphic microsatellite markers. Evidence of linkage was obtained on chromosome 6, with a peak 2-point logarithm of the odds (LOD) score of 3.63 (θ=0) at the marker D6S1021. A maximal multipoint LOD score of 4.9 was obtained between D6S286 and D6S1021, indicating odds of 100 000:1 in favor of this interval as the location of the gene defect responsible for AF in this family. The LOD scores were robust to changes in penetrance and allele frequency. Haplotype analyses further supported this minimal genetic interval.

Conclusion—We have mapped a novel locus for AF to chromosome 6q14–16. The identification of the causative gene in this interval will be an important step in understanding the fundamental mechanisms of AF. (Circulation. 2003;107:2880-2883.)

Key Words: arrhythmia • genetics • cardiomyopathy

Atrial fibrillation (AF) is the most common clinical arrhythmia, affecting >2 million Americans.1 AF presents a considerable socioeconomic burden, as it is associated with chronic medication use,1 nearly one fourth of all strokes in the elderly,2 and a reduced life expectancy in affected individuals.1 Most AF is considered secondary to other conditions such as hyperthyroidism, hypertension, left ventricular dysfunction, or valvular disease; however, a substantial minority of patients have no obvious cause for their condition and are said to have lone AF.3 The relationship between AF and structural heart disease is complex. It is known from animal models that the onset of the arrhythmia results in a series of adaptive and maladaptive responses, initiated within minutes.4,5 Dynamic changes in the expression of ion channels, proteins within the sarcoplasmic reticulum, and cytoskeletal elements have been described. Histological remodeling is evident within days.6 In a small series of patients with paroxysmal lone AF, there is evidence of an underlying myopathic or inflammatory process.7 Finally, the recent identification of a large subset of patients with lone AF in which the arrhythmia is initiated by pulmonary venous ectopy suggests a role for subtle structural or functional abnormalities below the current threshold for detection.8

In the last several years, the identification of “rare” kindreds with lone AF has led to genetic investigation of the primary causes of this arrhythmia. A genetic locus for AF has been described on chromosome 10q22 in 3 families, although the mutated gene has not yet been identified.9 Recently, in a single family in which the long-QT (LQT) syndrome and early-onset AF cosegregate, a gain-of-function mutation in the KCNQ1 gene has been reported.10 The genetic basis for the majority of AF cases remains obscure.

We have identified a large kindred (family FAF-1) in which AF segregates as a simple autosomal dominant trait (Figure 1). We have mapped the gene responsible for AF in this family to a locus on the proximal long arm of chromosome 6. This work demonstrates the extent of genetic heterogeneity in this arrhythmia and provides a basis for the cloning of the underlying gene defect.

Methods

Clinical Evaluations

All studies were performed with Institutional Review Board approval at Massachusetts General Hospital. Each family member had a physical examination and detailed history to identify the following: past medical conditions, medications, symptoms while in AF, and the medical history of all first-degree relatives. Each subject was
evaluated by 12-lead ECG, echocardiogram, and laboratory studies including one for thyroid-stimulating hormone. ECGs and echocardiograms were interpreted using standard criteria.

We defined as affected those individuals with electrocardiographically documented AF. We defined as unaffected only those individuals ≥60 years of age with no personal history of AF and no offspring with a history of AF. All other family members were classified as unknown for the purpose of initial genetic analyses.

Genetic Analyses
DNA was isolated from blood or Epstein-Barr virus–transformed lymphoblastoid cell lines. Genetic analyses were performed on all available individuals, regardless of affection status. Polymorphic genomic short-tandem repeat markers were used for mapping and were amplified by polymerase chain reaction using standard conditions. Genotypes were ascertained without knowledge of clinical status.

Statistical Analyses
Two-point and multipoint logarithm of the odds (LOD) scores were calculated assuming a disease penetrance of 0.95. Allele frequencies were estimated from the population. Two-point LOD scores were calculated with the MLINK program.11 To estimate the most likely location for disease, multipoint LOD scores were calculated using the program VITESSE,12 with the markers D6S286, D6S1021, and D6S404 and disease. The distances between loci were based on public data.

Results
Eight individuals in family FAF-1 were diagnosed with AF with a variable age of onset, ranging from 21 to 72 years (Table). There were no clinical features that distinguished the AF inherited in family FAF-1 from other forms of the arrhythmia. The QT interval was normal in all affected individuals. In the family, AF begins with paroxysmal episodes but becomes chronic in older individuals. In most affected subjects, echocardiography revealed mild left atrial dilatation with no evidence of structural heart disease. All affected family members were euthyroid. Individual 113 was

<table>
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<th>No.</th>
<th>Age of Onset, y</th>
<th>Current Age, y</th>
<th>Phenotype</th>
<th>Electrocardiogram</th>
<th>Left Atrium, mm</th>
<th>Ejection Fraction (at Presentation)</th>
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<tr>
<td>113*</td>
<td>59</td>
<td>61</td>
<td>Chronic AF</td>
<td>AF</td>
<td>44</td>
<td>0.71</td>
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<td>AF, NSSTTWA</td>
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<td>70</td>
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<td>Sinus rhythm</td>
<td>44</td>
<td>0.58</td>
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<tr>
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<td>38</td>
<td>0.64</td>
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<tr>
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<td>37</td>
<td>39</td>
<td>PPMP</td>
<td>Sinus rhythm</td>
<td>...</td>
<td>...</td>
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<tr>
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<td>28</td>
<td>36</td>
<td>PPMP</td>
<td>Sinus rhythm</td>
<td>28</td>
<td>0.60</td>
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</tbody>
</table>

NSSTTWA indicates nonspecific ST-segment and T-wave abnormality; PPMP, peripartum cardiomyopathy.
*Hypertension (see text).
†Congestive heart failure (see text).
The locus we have defined on chromosome 6q14–16 currently extends over 25 cM. On the basis of the locations of the recombinant boundaries, and the known structure of this region of the human genome, several putative candidate genes for AF may be proposed. We have already screened by direct sequencing the most obvious candidates, the genes encoding connexin 62, the 5-hydroxytryptamine receptor subtype 1E, and the thyroid receptor interacting protein TRIP7. We are currently defining the cardiac expression of other transcripts in the minimal interval to prioritize genes for subsequent screening. Analysis of the region reveals many other potential candidate genes. Interesting candidates include the thyroid-stimulating hormone α-polypeptide and, particularly given the incidence of sinoatrial dysfunction and AF in families with the LQT syndrome caused by ankyrin B mutations, the ankyrin repeat domain protein 6. Interest. Through our ongoing recruitment, we are identifying additional families that will allow us to refine the locus using new recombinants.

In common with the genetic interval previously defined for AF on 10q22,17 this region of chromosome 6q overlaps with a known locus for dilated cardiomyopathy,18 raising the possibility that lone AF and some forms of cardiomyopathy may be allelic. Additional support for this hypothesis comes from occasional families with monogenic forms of cardiomyopathy and a high prevalence of AF. Interestingly, at least 2 family members (individuals 18 and 264) who have inherited the disease haplotype in family FAF-1 have exhibited reversible left ventricular dysfunction. The cloning of the causative genes at the 10q22 and 6q14–16 loci will clarify the relationship between AF and cardiomyopathy.

Importantly, several individuals (individuals 171, 201, 212, 213, and 232) in the more recent generations of the family FAF-1 have inherited at least part of the disease haplotype but have no evidence of AF. These family members highlight the need for conservative phenotypic assignments in the genetic investigation of paroxysmal, or often asymptomatic, arrhythmias. The identification of the causative gene on chromosome 6q14–16 will impact the diagnosis and management of heritable AF, but may also illuminate the pathophysiology of secondary forms of the arrhythmia and those of related outcomes such as congestive heart failure and stroke.1

Discussion

These data strongly support a second locus for AF on the proximal long arm of chromosome 6 and confirm that this arrhythmia is genetically heterogeneous. Similar heterogeneity has been described for many other inherited cardiovascular disorders. The identification of the causative genes in such disorders has provided a unifying biological framework, often radically changing our understanding of the basic mechanisms of these conditions.13,14 Although an activating mutation in the KCNQ1 gene can cause AF in the context of the LQT syndrome, the role of this ion channel in typical clinical populations with AF remains to be explored.10 The cloning of the disease genes at other AF loci, such as those on chromosome 10q22 and now on chromosome 6q14–16, will be an important step in defining the fundamental mechanisms of this arrhythmia.9

diagnosed with hypertension at the time of his original presentation with AF. Individual 18 presented with evidence of congestive heart failure while in AF with a rapid ventricular response. An echocardiogram at that time revealed mild global hypokinesis, but all subsequent assessments of ventricular function have been normal. Two individuals, 263 (whose DNA is not available) and 264, have a history of peripartum cardiomyopathy but no AF, and were scored as unknown in all analyses.

We identified preliminary evidence of linkage with the marker D6S1595 (maximum LOD score = 2.30, \( \theta = 0 \)). Thirty-eight additional markers that have been mapped to chromosome 6 were subsequently analyzed. A peak 2-point LOD score of 3.63 (\( \theta = 0.10 \)) was achieved with marker D6S1021. This LOD score is robust to changes in penetrance or allele frequency, and even analyses of affected individuals only (scoring individual 264 and all other family members as unknown) result in 2-point LOD scores of >2.8. The peak multipoint LOD score of 4.9 (Figure 2) was obtained between markers D6S286 and D6S1021, indicating odds of \( \approx 100,000:1 \) in favor of this as the location of the gene defect responsible for AF in family FAF-1. Confidence intervals “1 LOD unit down” and haplotype analysis of critical recombinants within this region (Figure 1) further supported this as the disease gene location.11

Figure 2. Multipoint LOD score curve. Multipoint LOD score is plotted on the abscissa, and recombination distance (\( \theta \)) is plotted on the ordinate from an arbitrary origin set at marker D6S286.
Acknowledgments
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References
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