Mapping a novel locus for familial atrial fibrillation on chromosome 10p11-q21

Paul G.A. Volders, MD, PhD,*|| Qian Zhu, MD,†‡|| Carl Timmermans, MD, PhD,*
Petra M.H. Eurlings, PhD,‡ Xiaoyan Su, MD,† Yvonne H. Arens, MD, PhD,§ Li Li, MSc,‡
Roselie J. Jongbloed, PhD,§ Min Xia, MD,‡ Luz-Maria Rodriguez, MD, PhD,* Yi Han Chen, MD, PhD‡

From the *Department of Cardiology, Academic Hospital Maastricht, Maastricht, The Netherlands, the †Department of Cardiology, Tongji Hospital, and the §Institute of Medical Genetics, Tongji University, Shanghai, China, and the ¶Department of Clinical Genetics, Academic Hospital Maastricht, Maastricht, The Netherlands.

BACKGROUND Atrial Fibrillation (AF), the most common cardiac arrhythmia, is a significant public health problem in the United States, affecting approximately 2.2 million Americans. Recently, several chromosomal loci and genes have been found to be associated with familial AF. However, in most other AF cases, the genetic basis is still poorly understood.

OBJECTIVE The purpose of this study was to investigate the molecular basis of familial AF in a Dutch kindred group.

METHODS We analyzed a four-generation Dutch family in which AF segregated as an autosomal dominant trait. After the exclusion of linkage to 10q22-24, 6q14-16, 5p13, KCNQ1, KCNE2, KCNJ2 and some ion-channel-associated candidate genes, a genome-wide linkage scan using 398 microsatellite markers was performed.

RESULTS Two-point logarithms of odds (LOD) scores >1 at recombination fraction [θ] = 0.00 and a haplotype segregating with the disorder were demonstrated only across regions of chromosome 10. Subsequent fine mapping gave a maximum two-point LOD score of 4.1982 at D10S568 at [θ] = 0.00. Distinct recombination in several individuals narrowed the shared region among all affected individuals to 16.4 cM on the Genethon map (flanking markers: D10S578 and D10S1652), which corresponds to chromosome 10p11-q21. Thirteen candidate genes residing in this region, which could be associated with AF, were screened. No mutation has been found in their coding regions including the intron splice regions.

CONCLUSION We identify a novel locus for AF on chromosome 10p11-q21, which provides further evidence of genetic heterogeneity in this arrhythmia.

KEYWORDS Atrial fibrillation; Genetics; Mechanism

(Heart Rhythm 2007;4:469–475) © 2007 Heart Rhythm Society. All rights reserved.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia encountered in clinical practice, particularly in the elderly and in patients with organic heart diseases.1 The prevalence of AF is 0.4% in the general population and increases with age to up to 6%–8% in people over 80. There are 2.2 million affected people in the United States. AF is responsible for as many as 15% of the total number of strokes, compromised cardiac function, decreased quality of life, and a twofold increase in mortality. It leads to over 75,000 cases of stroke per year in the United States alone.2–5

In the past decade, we have witnessed extraordinary growth in all fields of knowledge regarding AF. Although the most widely accepted theory of its mechanism is Moe’s multiple wavelet hypothesis, other mechanisms, including focal origin, are also increasingly found to play a role.6 AF is generally classified as paroxysmal, persistent, and permanent.7,8 The management of AF today is directed toward the conversion to sinus rhythm, control of the ventricular rate, and the prevention of thromboembolism.9 Unlike other cardiac arrhythmias, there is still no highly successful therapy for AF,10 with the exception of patients presenting with focal mechanisms of AF based on abnormal impulse formation from the atria and pulmonary veins. These patients can be cured by catheter ablation.11,12

Coronary artery disease, hypertension, heart failure, and valvular heart disease are common underlying substrates for the arrhythmogenesis of AF. The arrhythmia may also occur...
without obvious structural heart disease (“lone AF”).1 A recent survey in a large cohort of lone AF demonstrated that 39% of individuals had a positive family history.13 The latest epidemiological data from the Framingham study in an unselected population–based sample showed that 30% of AF patients had a genetic background, greatly beyond the amount that we previously thought. Parental AF independently increases the risk of future offspring AF events.14 Three defective potassium channel genes, \( KCNQ1 \) (S140G),15 \( KCNE2 \) (R27C),16 and \( KCNJ2 \) (V93I),17 were identified in Chinese families with AF. In addition, three other loci on chromosomes 10q22-24,18 6q14-16,19 and 5p1320 have been reported, with no defective genes yet identified. Recently, a de novo gain-of-function mutation in \( KCNQ1 \) (V141M) that is responsible for AF and short QT syndrome in utero has been reported.21 Also, sodium channel gene (\( SCN5A \)) defects were associated with a higher susceptibility to early-onset dilated cardiomyopathy (DCM) and AF.22 A loss-of-function mutation of ANK-B (ANK2) was identified in an extended kindred with long QT syndrome (LQTS), sinus node dysfunction, and AF.23 Moreover, mutations in the nuclear membrane protein lamin A/C (LMNA) have pleiotropic noncardiac and cardiac manifestations including DCM, AF, and conduction system disease.24 Taken together, AF is a potentially heterogeneous disorder, and more genetic defects remain to be identified.25

In the present study, we mapped a novel genetic locus for AF on chromosome 10p11-q21 in a four-generation Dutch family using genome-wide linkage analyses.

**Methods**

**Clinical evaluations**

A four-generation Dutch family with AF was enrolled in the study (for pedigree see Figure 1). All family members and spouses were evaluated by a thorough clinical history (including previously diagnosed cardiac conditions, syncope, palpitations, and medications), physical examination, standard 12-lead electrocardiograms (ECGs), echocardiograms, 48-hour ambulatory ECGs, and/or clinical electrophysiological examination. Serial ECGs were performed since 1997 and before hospital records were obtained when available. For family members who had died, surviving relatives were questioned and hospital records were examined. The diagnostic criteria for AF were based on the ECG as follows: (1) absence of distinct, regular P waves, (2) irregular atrial electrical activity of 350–600/minute, and/or (3) irregular ventricular rhythm. Organic heart disease and other causative factors, such as hypertension, coronary artery disease, valvular heart disease, congenital heart disease (other than familial AF), cardiomyopathy, congestive heart failure, pericarditis, diabetes mellitus, chronic obstructive pulmonary disease, and hyperthyroidism were reasons for exclusion. Individuals without any proof of AF based on the above examinations and criteria were classified as “unaffected.” Individuals with any sign (even one) of AF, and not having organic heart disease, were classified as “uncertain.”

The investigation conformed with the principles outlined in the Declaration of Helsinki and was approved by the Ethical Committee at Tongji University School of Medicine, Shanghai, China, and the Medical Ethics Committee of the Academic Hospital Maastricht, The Netherlands. Written informed consent was obtained from the adult individuals and the parents of the adolescents. Adolescents also agreed to participate in the study.

**DNA samples and mutation analyses**

Genomic DNA was extracted from peripheral blood lymphocytes with Wizard Genomic DNA Purification Kit (Promega, USA). Previously identified AF causative genes, \( KCNQ1, KCNE2, \) and \( KCNJ2 \), were screened for mutations. Two affected (II:13 and III:1) individuals were chosen for mutation screening. In addition, we also analyzed the coding and intron splice sequences of several ion-channel genes and ion-channel-associated genes (\( ANK-B, HERG, KCNE1, KCNE3, KCNE4, KCNE5, SCN5A \) and \( LMNA \)). During the

![Figure 1](http://example.com/figure1.png)
candidate-gene study within chromosome 10p11-q21, two ion-channel-related genes PCDH15 and ANK3 isoform 2 and the CX40.1, NRBF2, TFAM, FXD4, NCOA4, ANXA8, CXCL12, PRKG1, UBE2D1, CDC2, and EGR2 genes were selected to be sequenced (data information from www.ncbi.nlm.nih.gov/entrez and www.genecards.org). Using Primer-3 software (Whitehead Institute for Biomedical Research, USA), we designed the primers of all the coding regions including the intron splice regions of genes. Subsequent analyses were performed by polymerase chain reaction (PCR)-direct sequencing, using the DYEnamic ET dye terminator kit (Amersham Biosciences, UK Limited), and sequences were analyzed on MegaBACE 500 (Amersham Biosciences Inc, USA).

Genotyping and linkage analyses
After KCNQ1, KCNE2, and KCNJ2 were excluded, three known AF loci (chromosomes 5p13, 6q14-16, and 10q22-24) were screened via genetic linkage analysis using their flanking microsatellite markers. Finally, genome-wide scanning was performed. A total of 398 microsatellite markers (ABI PRISM Linkage Mapping Sets v2.5-MD-10, Applied Biosystems, USA) at an even density of 10 cM from chromosomes 1 to 22 were genotyped in 32 family members. Multiplex PCR was done by using three or four genome scan markers with Ampli Taq Gold (PE Applied Biosystems, USA) at an even density of 10 cM from chromosomes 1 to 22 were genotyped in 32 family members. Multiplex PCR was done by using three or four genome scan markers with Ampli Taq Gold (PE Applied Biosystems, USA) at an even density of 10 cM from chromosomes 1 to 22 were genotyped in 32 family members. The PCR products were separated on an automatic ABI 3100 Genetic Analyzer. Genotyping data were scored using the GeneMapper 2 (Applied Biosystems, USA) without the scorer having knowledge of the phenotypes. When a supportive two-point logarithms of odds (LOD) score was found on chromosome 10, eight additional microsatellite markers were selected to map finely. The mean distance between two markers was 1.5 cM. Marker order and distances were obtained from the Genethon map. Two-point LOD scores were calculated using the program SAGE 5.0 with the frequency and penetrance of AF set at $10^{-3}$ and 95%, respectively. Haplotype analysis was carried out manually. All the genotypes were obtained independently from phenotypic information.

Results
Clinical characteristics of the pedigree
In the four-generation family, AF segregated as an autosomal dominant trait (Figure 1). The family originated from the province of Limburg in the south of The Netherlands. In total, 53 family members and spouses were involved in the study, 34 of whom provided DNA samples. There were six affected with documented AF on the ECG. I:1 died acutely at home in 1952 (at the age of 54), probably of cardiovascular cause. His past medical history stated an irregular pulse during the last period of his life. Based on this information, we have reason to speculate that I:1 had AF. However, in the absence of an ECG showing AF, his phenotype was still classified as “uncertain.” I:2 died of cancer (uterus carcinoma) at the age of 65 without any sign of AF. The proband (III:7; Figure 1; Table 1) was coincidentally diagnosed with paroxysmal AF at the age of 41 when her ECG was recorded for occupational reasons. Although fatigue and decreased exercise tolerance had limited her abilities, these complaints had not previously prompted her to see a

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical data of carriers of the disease haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient no.</td>
<td>Sex</td>
</tr>
<tr>
<td>I:1</td>
<td>M</td>
</tr>
<tr>
<td>II:1</td>
<td>F</td>
</tr>
<tr>
<td>II:2</td>
<td>M</td>
</tr>
<tr>
<td>II:3</td>
<td>M</td>
</tr>
<tr>
<td>II:4</td>
<td>F</td>
</tr>
<tr>
<td>II:5</td>
<td>F</td>
</tr>
<tr>
<td>II:6</td>
<td>F</td>
</tr>
<tr>
<td>II:7</td>
<td>F</td>
</tr>
<tr>
<td>II:8</td>
<td>F</td>
</tr>
<tr>
<td>II:9</td>
<td>F</td>
</tr>
</tbody>
</table>

| LAD = left atrial diameter; LVEDD = left ventricular end-diastolic diameter; LVEF = Left ventricular ejection fraction; LVESD = left ventricular end-systolic diameter; NA = not available or not applicable; QTc = corrected QT interval; SVEs = supraventricular extrasystoles. |

*Age at death.
physician. She had episodes of mild chest discomfort, palpitations, and dizziness in the recent past. At the present time, III:7 has persistent AF. Representative 12-lead ECGs during sinus rhythm and AF at the moment of diagnosis are shown in Figures 2A and 2B. During clinical electrophysiological study in the proband at age 51, intracardiac recordings revealed that the site of earliest electrical activation was in the left common pulmonary vein (Figure 3). Ectopic impulses from this region (at a cycle length of approximately 180 ms) were capable of triggering AF.

In affected individuals, the mean age of onset of AF was 51 ± 3 (range 40–58) years. In four family members (II:2, II:13, III:1, and III:3), AF was already persistent at the time of diagnosis and presently is permanent. None had organic heart disease, and none were taking any medication at that time. In two members (III:1 and III:3), transient cerebral ischemic attacks (TIAs) preceded the recognition of the cardiac arrhythmia. Whereas the clinical symptoms disappeared in both individuals within 1 day, a limited pathological substrate remained demonstrable by computed tomography imaging of the brain (2 weeks later): lacunar infarcts of the right paraventricular region (III:1) and of the left internal capsule and paraventricular region (III:3). The concomitant occurrence of TIAs and de novo AF of unknown
duration in both patients’ lives led us to assume that a left-atrial origin of the thromboemboli was the most likely after other risk factors had been excluded. In III:19, the youngest among the affected family members, AF is paroxysmal. The clinical data are provided in Table 1.

In all affected persons, retrospective analysis of the last available ECGs during sinus rhythm did not reveal abnormalities of the P wave, atrioventricular conduction, QRS complex, or T-wave morphology (see also Figure 2). Trans-thoracic echocardiography showed normal left-atrial and left-ventricular size (Table 1), normal septal and posterior left-ventricular wall thickness, normal left-ventricular ejection fractions (>55%), and normal heart valves. Interestingly, three younger family members (IV:2, aged 30; IV:6, aged 21; and IV:14, aged 16), who had a positive haplotype, had incidental supraventricular salvos and/or frequent single supraventricular extrasystoles (SVEs) on their 48-hour ambulatory ECGs, along with complaints of palpitations and chest discomfort, but no recorded AF (see Table 1). An example of a supraventricular salvo (IV:2) is shown in Figure 2C. The average rate of these salvos was 135 ± 12 (range 114–156) bpm. The phenotypes of IV:2, IV:6, and IV:14 were set as “uncertain” for linkage analysis because they were considered abnormal but AF had not been demonstrated. Supraventricular salvos were not observed in unaffected individuals III:10, III:12, III:14, III:16, III:21, IV:1, IV:3, IV:4, IV:5, IV:12, IV:13, and IV:16, who had no or only very low counts of SVEs (ranging from 0 to 37 SVEs per individual per 24 hours). Their phenotypes were set as “unaffected” (for criteria, see the Methods section).

Mapping of a novel locus for familial AF to chromosome 10p11-11q21
First, we excluded the presence of previously reported AF causative genes or AF-associated loci and other candidate genes. We sequenced all the exons of three cardiac K⁺-channel genes KCNQ1, KCNE2, and KCNJ2, which are known to lead to AF, from affected individuals II:13 and III:1. No mutation was found. Subsequently, previously identified AF loci on chromosomes 5p13, 6q14-16, and 10q22-24 were examined using flanking markers (D5S455, D5S1998, D6S1601, D6S1595, D6S1021, D10S537, and D10S1686). Two-point LOD scores were less than −2, and therefore these loci were excluded as candidate loci. Moreover, mutation analyses revealed no abnormalities in the coding sequences of other candidate genes including ANK-B, HERG, KCNE1, KCNE3, KCNE4, KCNE5, SCN5A, and LMNA.

After genome-wide scanning at a density of 10 cM, a two-point LOD score of 3.9645 at D10S1652 was preliminarily obtained. Then additional microsatellite markers D10S578, D10S220, D10S568, D10S539, D10S1790, D10S1756, D10S589, and D10S1681 (mean density of 1.8 cM; heterozygosities >70%) were genotyped for fine mapping. A peak two-point LOD score of 4.1982 was obtained with D10S568. Generally speaking, an LOD ≥3 is the standard for “establishing linkage,” that is, rejecting the null hypothesis of no linkage.26 The higher the LOD score, the stronger the support of linkage. The locus was ultimately mapped to a 16.4-cM interval between D10S578 and D10S1652 on chromosome 10p11-q21 through haplotype analysis (Figures 1 and 4; Table 2). Two-point LOD scores remained >3 despite variations in penetrance from 60 to 99 percent and variations in the phenocopy prevalence from 0 to 5 percent.

Since the novel locus was also on chromosome 10, despite an at least 20-cM distance from the previously identified locus 10q22-24,18 we selected five additional microsatellite markers (D10S210, D10S569, D10S607, D10S1677, and D10S1652), approximately at a density of 2 cM, and this resulted in LOD scores <−2, which rules out the potential linkage between chromosome 10q22-24 and AF in this Dutch pedigree.

Identification and screening of candidate genes within the critical region
We screened the newly identified locus on chromosome 10p11-q21 for candidate genes. The mutational screening within the critical region was focused on the evident genes based on their cardiac expression and suspected function. First, two obvious candidates were ankyrin 3 isoform 2 (ANK3) and protocadherin 15 (PCDH15). Analysis of the coding and intron splice sequences of both genes in affected subjects II:13, III:1, with unaffected III:8 as the control,
failed to identify any mutation. Only some single nucleotide polymorphisms (SNPs) were found, three in ANK3 and two in PCDH15, which have previously been reported (http://www.ncbi.nlm.nih.gov/SNP).

Other candidate genes within the region are connexin 40.1 (Cx40.1), nuclear receptor binding factor 2 (NRBF2), mitochondrial transcription factor A (TFAM), type I cGMP-dependent protein kinase (PRKG1), nuclear receptor coactivator 4 (NCOA4), annexin A8 (ANXA8), chemokine ligand 12 (CXCL12), FXYD domain-containing ion transport regulator 4 (FXYD4), ubiquitin-conjugating enzyme E2D 1 (UBE2D1), cell division cycle 2 (CDC2), and early growth response 2 (EGR2) genes. However, no mutation was found in the coding and intron splice sequences of these genes.

Discussion

By linkage analysis, we have mapped a novel locus for familial AF to chromosome 10p11-q21 in a 16.4-cM interval between D10S578 and D10S1652. The finding of this novel locus further confirms the genetic heterogeneity of familial AF, as indicated previously by us and other investigators.15–22

In most affected members of the Dutch pedigree of this study, AF produced only mild symptoms and the arrhythmia was hemodynamically well tolerated. While these features appear benign by themselves, the failure to diagnose and terminate AF early after its initiation confers a serious risk of thromboembolic complications. Indeed, TIAs preceded the clinical recognition of AF in two Dutch family members. It is very likely that left-atrial thrombi caused these cerebrovascular events. The finding of novel genetic markers of AF, as in this and previous other studies, may expand our arsenal for screening out persons at risk.

No mutation was identified in three AF causative K⁺-channel genes, KCNQ1, KCNE2, and KCNJ2, in the Dutch family. To further investigate the role of ion-channel genes and ion-channel-associated genes in the pathogenesis of AF for the Dutch family, we collected all the expressed sequence tag (EST) uniclusters information within the interval between D10S578 and D10S1652 (www.ncbi.nlm.nih.gov/mapview) and obtained 48 clusters total. Based on tissue expression and suspected function of these clusters, we sorted out the uniclusters involved in ion or ion-channel-associated genes and obtained five unigenes (Hs.40861, Hs.232819, Hs.440478, Hs.438237, and Hs.436623). The former two unigenes were overlapping with PCDH15, and the latter three with ANK3 isoform 2, for which no mutation had been found. The above work was carried out manually (data not shown). Thus, it is possible for non-ion-channel genes to be candidates for AF in the Dutch family. For three other chromosomal loci 10q22-24, 6q14-16, and 5p13 for AF,18–20 no mutation of ion-channel genes has been implicated.

Within the 16.4-cM region on chromosome 10p11-q21, candidate genes for non-ion-channel genes include ankyrin 3 (ANK3) and protocadherin 15 (PCDH15). No mutation was found in ANK3 isoforms and PCDH15 except in five reported SNPs (http://www.ncbi.nlm.nih.gov/SNP). Likewise, sequencing analysis of Cx40.1, also located on 10p11.21, which encodes for an integral part of the connexin complex, did not reveal any mutation.

Other sophisticated cellular processes also influence ion-channel function. These include biophysical (e.g., gating, permeation), biochemical modulation (e.g., phosphorylation, glycosylation) of channel subunits, and biogenic modulation (e.g., biosynthesis, processing, trafficking, degradation).27 We selected 10 more genes associated with the above cellular processes: NRBF2, TFAM, FXYD4, NCOA4, ANXA8, CXCL12, PRKG1, UBE2D1, CDC2, and EGR2. No mutation was identified in the coding and intron splice sequences of these genes.

Study limitation

A significant proportion of patients with AF, notably paroxysmal AF, can be asymptomatic, thus possessing ”silent” positive phenotypes. In such patients, the phenotype could have been missed by clinical screening. We cannot rule out that this has affected the linkage analyses.

Conclusions

A novel locus for familial AF was mapped to chromosome 10p11-q21. After analyzing the coding and intron splice

Table 2 Two-point LOD scores

<table>
<thead>
<tr>
<th>Marker</th>
<th>.00</th>
<th>.01</th>
<th>.05</th>
<th>.10</th>
<th>.20</th>
<th>.30</th>
<th>.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>D10S1780</td>
<td>(−)</td>
<td>−0.6512</td>
<td>0.5419</td>
<td>0.8759</td>
<td>0.8975</td>
<td>0.6090</td>
<td>0.2207</td>
</tr>
<tr>
<td>D10S578</td>
<td>2.7154</td>
<td>2.6673</td>
<td>2.4698</td>
<td>2.2109</td>
<td>1.6473</td>
<td>1.0145</td>
<td>0.3570</td>
</tr>
<tr>
<td>D10S220</td>
<td>3.9134</td>
<td>3.8479</td>
<td>3.5792</td>
<td>3.2270</td>
<td>2.4598</td>
<td>1.5927</td>
<td>0.6358</td>
</tr>
<tr>
<td>D10S568</td>
<td>4.1982</td>
<td>4.1286</td>
<td>3.8627</td>
<td>3.4679</td>
<td>2.6518</td>
<td>1.7283</td>
<td>0.6984</td>
</tr>
<tr>
<td>D10S539</td>
<td>(−)</td>
<td>−0.3897</td>
<td>0.2259</td>
<td>0.4133</td>
<td>0.4584</td>
<td>0.3489</td>
<td>0.1790</td>
</tr>
<tr>
<td>D10S1790</td>
<td>3.7885</td>
<td>3.7244</td>
<td>3.4619</td>
<td>3.1179</td>
<td>2.3698</td>
<td>1.5278</td>
<td>0.6131</td>
</tr>
<tr>
<td>D10S1756</td>
<td>4.0895</td>
<td>4.0211</td>
<td>3.7406</td>
<td>3.3732</td>
<td>2.5737</td>
<td>1.6710</td>
<td>0.6700</td>
</tr>
<tr>
<td>D10S589</td>
<td>2.7885</td>
<td>2.7432</td>
<td>2.5552</td>
<td>2.3039</td>
<td>1.7444</td>
<td>1.1083</td>
<td>0.4342</td>
</tr>
<tr>
<td>D10S1652</td>
<td>3.9645</td>
<td>3.8976</td>
<td>3.6232</td>
<td>3.2640</td>
<td>2.4836</td>
<td>1.6051</td>
<td>0.6407</td>
</tr>
<tr>
<td>D10S581</td>
<td>(−)</td>
<td>0.9209</td>
<td>1.4243</td>
<td>1.4719</td>
<td>1.2347</td>
<td>0.8273</td>
<td>0.3390</td>
</tr>
<tr>
<td>D10S210</td>
<td>(−)</td>
<td>−1.2079</td>
<td>0.0175</td>
<td>0.3944</td>
<td>0.5142</td>
<td>0.2207</td>
<td>0.2207</td>
</tr>
</tbody>
</table>
regions of the 13 candidate genes within the locus, identification of the responsible gene is pending. This study should shed more light on the mechanism of this genetic form as well as on the common acquired forms of the arrhythmia.

Acknowledgments
We are indebted to all family members for participating in the study. The authors are grateful to Mrs. Suzanne A.M. Phillipps and Mr. Roel L.H.M.G. Späjtjens, Department of Cardiology, and Mrs. Dimphy A.M. Huveners-Reniers, Department of Clinical Genetics, Academic Hospital Maastricht, The Netherlands, for their help with the data collection.

References