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Comprehensive Mutation Scanning of *LMNA* in 268 Patients With Lone Atrial Fibrillation

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Abstract

Atrial fibrillation (AF) is a heritable, genetically heterogeneous disorder. To identify gene defects that cause or confer susceptibility to AF, a cohort of 268 unrelated patients with idiopathic forms of familial and sporadic AF was recruited. *LMNA*, encoding the nuclear membrane proteins, lamin A/C, was selected as a candidate gene for lone AF based on its established association with a syndrome of dilated cardiomyopathy, conduction system disease, and AF. Comprehensive mutation scanning identified only 1 potentially pathogenic mutation. In conclusion, *LMNA* mutations rarely cause lone AF and routine genetic testing of *LMNA* in these patients does not appear warranted.

LMNA encodes the ubiquitously expressed intermediate filament proteins lamin A/C. The alternatively spliced gene products localize to the nucleus, where they confer structural integrity to the inner nuclear membrane and influence gene expression. Heterozygous mutations in *LMNA* have pleiotropic effects and can cause a spectrum of distinct, yet sometimes overlapping, disorders, including striated muscle diseases, peripheral neuropathy, partial lipodystrophy syndromes, and premature aging syndromes.¹ However, pathogenic mechanisms for laminopathies are poorly understood. *LMNA* has been identified as a disease gene for a clinical subtype of dilated cardiomyopathy associated with conduction system disease, implicating a role for lamin A/C in cardiac muscle integrity and electrical stability. Atrial fibrillation (AF) is frequently observed in patients with *LMNA*-associated dilated cardiomyopathy.^{2–5} We postulated that mutations in *LMNA* could underlie some cases of idiopathic AF not associated with a cardiomyopathic phenotype. By analogy, we recently reported a case of familial AF without muscular dystrophy caused by a cardioselective mutation in *EMD*, encoding the nuclear membrane protein emerin.⁶ Similarly, mutations in *SCN5A*, encoding the cardiac sodium channel, have been identified in families with a syndrome of dilated cardiomyopathy and AF⁷ and those with isolated AF.⁸

Methods

Subjects were recruited from the Mayo Clinic, Rochester, Minnesota, and National University Hospital, Singapore, under approved research protocols after obtaining informed written

consent. Inclusion criteria were documented AF in subjects <60 years of age who lacked known risk factors, including hypertension and structural heart disease. The study cohort was composed of 268 unrelated subjects (78% men) with a mean age of 44.8 years at diagnosis. Racial subgroups included 72% white, 22% Asian, 1% American Indian, and 5% unknown. Familial AF, defined as idiopathic AF in ≥ 1 relative, was documented in 40%. Genomic DNA was isolated from peripheral- blood white blood cells of study subjects using a Puregene Blood Kit (Gentra/Qiagen, Valencia, California).

Primer pairs for polymerase chain reaction amplification of the 12 exons and ≥ 50 base pairs of the flanking intron sequence of *LMNA* were designed using Oligo Primer Analysis Software, version 6.71 (Molecular Biology Insights, Cascade, Colorado). Amplified products were screened for sequence variants by denaturing high-performance liquid chromatography (DHPLC) heteroduplex analysis using the Wave DHPLC System (Transgenomic, Omaha, Nebraska). Ideal buffer gradients and column-melting temperatures were determined using Transgenomic Navigator software, version 1.7.0, Build 25, and subsequent optimization. Primer sequences, amplicon sizes, and melting temperatures used in mutation detection for each exon are listed in Table 1. Each sample that showed a variant profile was sequenced using the dye-terminator method with an ABI Prism 3730 XL DNA Analyzer (Applied Biosystems, Foster City, California).

The Ensembl (ensembl.org/index.html), Leiden Open Variation Database (LOVD; www.dmd.nl/nmdb/home.php), and National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) online databases were queried for identified sequence variants. Potential effects on messenger RNA splicing were analyzed using Genscan (www.genes.mit.edu/GENSCAN.html) and NetGene2 (cbs.dtu.dk/services/NetGene2). Conservation of amino acids altered by missense variants was investigated by aligning human *LMNA* to chimpanzee, dog, cow, mouse, and rat *LMNA* using the HomoloGene and BLASTP links on the National Center for Biotechnology Information Web site. Samples from ethnically matched control subjects were screened using DHPLC for each DNA sequence variant that altered protein sequence or was predicted to affect splicing. Annotation for sequence variants was in the same format as LOVD and was based on Reference: sequences NC_000001.9 (genomic DNA), NM_170707.2 (complementary DNA), and NP_733821.1 (protein).

Results

Results are listed in Table 2. Data were not shown for several common previously reported synonymous and intronic single-nucleotide polymorphisms considered to be biologically neutral. Eight additional identified variants were analyzed further. Two unreported heterozygous intronic variants were identified in a familial case (c.810+63C→A) and a sporadic case (c.937-46A→G). Neither was predicted to create a cryptic splicing site, and the former variant did not segregate with familial AF. In addition, the same heterozygous intronic variant (c.1158-44C→T) was identified in 2 apparently unrelated white subjects with sporadic disease. This variant was previously identified in a patient with dilated cardiomyopathy and AF and deposited in LOVD (LMNA_00169). Moreover, it was predicted to potentially create a cryptic splice site using the NetGene2 analytical program (donor splice site confidence level 0.56). However, the variant was also present in 2 controls, indicating that it was likely a benign polymorphism. Two unreported heterozygous synonymous variants were found in 3 sporadic cases (E383E and S601S). Neither was predicted to create a cryptic splicing site.

Three unreported heterozygous nonsynonymous variants were identified in 2 sporadic cases. A sample from an Asian subject had 2 missense variants, each resulting in a conservative amino acid substitution (G125S and V415I). The same mutation pair was also found in 1 ethnically matched control, suggesting that they represent a rare nonpathogenic haplotype. A sample from

a white man had a missense mutation (T488P) resulting in substitution of a conserved aliphatic residue with an aromatic residue in the tail domain of both lamin isoforms. He had a normal echocardiogram and no atrioventricular block. This variant was absent in 360 control samples and to our knowledge has never been reported. The family history was negative for AF, but relatives declined to undergo clinical and genetic screening, precluding segregation analysis.

Discussion

High-throughput systems for mutation screening based on altered melting properties of variant DNA sequences provide an efficient, sensitive, and cost-effective approach to screen for mutations in candidate genes for heritable cardiovascular disorders.^{9–11} Such techniques have been used to identify novel genes for AF⁹ and determine mutation frequency for established AF genes¹⁰ in large patient cohorts. Detection sensitivity of 92.5% to 100% has been reported for DHPLC analysis versus comprehensive dye-terminator sequencing in other gene mutation screens.¹² To ensure optimal sensitivity, we used 1 to 3 melting temperatures for each *LMNA* amplicon based on Navigator software advanced melt profiles of amplified DNA sequence. Using DHPLC, we did not find conclusive evidence for pathogenic mutations in *LMNA* in a large cohort of patients with lone AF. A pathogenic role for a single previously unreported T488P substitution was implicated, but not proved. Although our findings did not exclude the possibility that *LMNA* mutations could rarely cause isolated lone AF, routine genetic testing of *LMNA* in these patients does not appear warranted.

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Table 1
Denaturing high-performance liquid chromatography conditions

Exon	Size (base pairs)	Forward Primer (5'→3')	Reverse Primer (5'→3')	Melting Temperature(s) (°C)
1a	337	AGATCCCGAGGTCCGACAGC	TTCTCCGTTTCCAGCGAGCG	65.5, 68
1b	333	CGCTCGCTGAAACGGAGAAC	AGGAGACTATGGCAAAGTTATCGG	63, 66.5
2	346	TGCCCTCTCCTGGTAATTG	TAGGTAGAAGAGTGAGTGTA	60.5, 62.5, 63
3	229	CTTGTGTTCTGTGACCCCTT	CCAAGTCTGTCATCACCCA	61.7
4	341	GCCCTGGGTCTTGCCCTCC	GCCACCATCTGCCTGATCC	61, 63.5
5	231	CCTCCACCCCTCCCAGTCAC	TGCATCCGGCCCAGACTCTAG	63.5, 64.5
6	372	TGTCTCCTACACCGACCCACG	GGGTCTAGTCAAGGCCAGT	63.7
7	358	GAGGTGCTGGCAGTGTCTC	CTTCTGTCTTGCCACTCTCTCC	63.5, 65
8	262	GGGCCTTTGAGCAAGATACA	AGAGGAGAAAAGGACACTTA	62.5
9	337	GATGATCCCTTGCTGACTTA	GCTGGCTCCGATGTTG	63.8
10	324	AGGTGGTCACTGGGGTAGAC	GGGAGGAGAGAGAAGAAAGG	58, 63.5, 65
11	370	AGCCTGGAGCCTGGTTG	TGGAGACAAAGCAGAGAC	64.7, 65.5, 66
12	197	GTGAGGGATGGGGGAGATGC	GGGTGGGCATGAGGTGAGG	61.8

Table 2
LMNA sequence variants identified in patients with atrial fibrillation

Identification	Exon/Intron	Variant	Amino Acid	Conserved*	Cryptic Splicing Predicted	Race	Controls With Variant	Familial
A564	2	c.373G→A	G125S	Yes	No	Asian	1/180	No
A150	IVS4	c.810+63C→A	NT	NT	No	White	NT	Yes
A129	IVS5	c.937-46A→G	NT	NT	No	White	NT	No
A550, A563	6	c.1149G→A	E383E	NT	No	Asian	NT	No
A58, A123	IVS6	c.1158-44C→T	NT	NT	Yes	White	2/180	No
A564	7	c.1243G→A	V415I	No [†]	No	Asian	1/200	No
A165	8	c.1462A→C	T488P	Yes	No	White	0/360	No
A498	11	c.1803C→T	S601S	NT	No	White	NT	No

* Invariant residue in chimpanzee, dog, cow, mouse, and rat.

[†] 1415 in dog.

NT = not tested or not relevant.