

Association of Rare Genetic Variants and Early-Onset Atrial Fibrillation in Ethnic Minority Individuals

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IMPORTANCE Although rare variants in cardiac ion channels, transcription factors, and myocardial structural proteins are associated with early-onset atrial fibrillation (AF) in White individuals of European descent, it remains unclear whether genetic variation also contributes to the cause of AF in those of minority ethnicity.

OBJECTIVES To assess the prevalence of rare and novel pathogenic variants in candidate genes in ethnic minority probands with early-onset AF and determine genotype-phenotype associations.

DESIGN, SETTING, AND PARTICIPANTS In this cohort, family-based study, probands of African and Hispanic descent with early-onset AF (defined as AF occurring in individuals aged ≤ 66 years) prospectively enrolled in a clinical and genetic biorepository underwent sequencing of 60 candidate genes. Recruitment took place from July 1, 2015, to June 30, 2019. Data were analyzed from February 1 to February 28, 2020.

EXPOSURES Rare and novel variants categorized as pathogenic or likely pathogenic.

MAIN OUTCOMES AND MEASURES The prevalence of rare and novel pathogenic variants in African American and Hispanic/Latinx probands with early-onset AF and genotype-phenotype associations.

RESULTS Among 227 probands with early-onset AF, mean (SD) age at onset of AF was 51.0 (9.9) years, 132 probands (58.1%) were men, 148 (65.2%) were African American, and 79 (34.8%) were Hispanic/Latinx. A family history of AF was verified in 24 probands with early-onset AF (10.6%). Sequencing 60 candidate genes identified 53 (23 rare and 30 novel) variants with 16 of the 227 (7.0%) probands harboring likely pathogenic (43.8%) or pathogenic (56.2%) variants, with most loss-of-function variants in *TTN*, the gene encoding the sarcomeric protein titin (46.7%). In 6 families with more than 2 affected members, variants of unknown significance in sodium channel (*SCN10A*), potassium channel (*KCNES*), sarcomeric proteins (*MYH6* and *TTN*), and atrial natriuretic peptide (*NPPA*) cosegregated with AF.

CONCLUSIONS AND RELEVANCE In this study, likely pathogenic and pathogenic variants were identified, with most loss-of-function variants in *TTN*, that increase susceptibility to early-onset AF in African American and Hispanic/Latinx individuals. These findings provide further understanding toward molecular phenotyping of AF and suggest novel mechanism-based therapeutic approaches for this common arrhythmia in ethnic minority groups.

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Genetic approaches to the mechanisms of atrial fibrillation (AF), including positional cloning, candidate gene sequencing, and genome-wide association studies, have identified variants in cardiac ion channels, transcription factors, and myocardial structural proteins in White individuals of European descent and provided insights into the underlying pathophysiologic factors.¹ It is established that non-White individuals are at a lower risk of developing AF, especially African American and Hispanic/Latinx individuals despite a greater burden of cardiovascular risk factors and poorer outcomes.²⁻⁴ Over the past 2 decades, several studies have identified rare genetic variants associated with early-onset AF in White individuals, but the contribution of rare pathogenic variants to the source of the arrhythmia in ethnic minority groups remains unclear.⁵⁻⁷ Two studies from 2018 reported that probands of African and Hispanic descent with early-onset AF were more likely to have a first-degree relative with AF compared with White individuals.^{8,9} Herein, we describe comprehensive sequencing of 60 candidate AF genes in African American and Hispanic/Latinx probands to examine the combined prevalence of rare pathogenic variants and assess genotype-phenotype association.

Methods

We recruited patients with early-onset AF and their family members from University of Illinois at Chicago and Jesse Brown Veterans Administration Medical Centers, with all probands self-reported as being of African or Hispanic descent and older than 18 years at the time of enrollment with a history of AF documented by an electrocardiogram, Holter monitor, or implantable loop recorder. Recruitment took place from July 1, 2015, to June 30, 2019. Data were analyzed from February 1 to February 28, 2020. More than 75% of the Hispanic/Latinx cohort are individuals of Mexican descent, which is consistent with the Hispanic population in Chicago. Patients with a history of AF associated with cardiothoracic surgery were excluded. We obtained information on demographics, cardiovascular risk factors, and family history for baseline characteristics with blood drawn for DNA extraction at the time of enrollment as previously reported.^{8,9} Experienced English- and Spanish-speaking research coordinators obtained a family history and constructed a family pedigree when a proband gave a family history of AF or stroke in a first-degree relative. We then contacted the family member, enrolled them in the registry, and reviewed their medical records, and AF was confirmed by electrocardiogram, Holter monitor, or event recorder.

We obtained written informed consent from all participants under a protocol approved by the University of Illinois at Chicago and the Jesse Brown Veterans Administration institutional review boards. Bilingual research coordinators recruited patients using both English and Spanish consent forms. Participants did not receive financial compensation, and the data were deidentified as stipulated by the institutional review boards. This study followed the Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

Key Points

Question What is the prevalence of rare likely pathogenic or pathogenic variants in candidate genes in ethnic minority probands with early-onset atrial fibrillation and genotype-phenotype associations?

Findings In this cohort study, a family history of atrial fibrillation was noted in 24 (10.6%) African American and Hispanic/Latinx probands with early-onset atrial fibrillation; sequencing identified 16 (7.0%) probands harboring pathogenic (56.2%) or likely pathogenic (43.8%) variants, with most (46.7%) loss-of-function variants in the *TTN* gene. In 6 families with more than 2 affected members, variants of unknown significance in cardiac ion channels, sarcomeric proteins, and signaling molecules cosegregated with atrial fibrillation.

Meaning Likely pathogenic or pathogenic variants with most loss-of-function variants in *TTN* that increase susceptibility to atrial fibrillation were observed in African American and Hispanic/Latinx individuals; these findings may provide insights into the underlying pathophysiologic factors and potential mechanism-based therapies for atrial fibrillation in individuals of minority ethnicity.

We defined early-onset AF as AF with onset when the participants were 66 years or younger and familial AF as the presence of AF in 2 or more first-degree relatives of probands with early-onset AF. Rare variants were defined as minor allele frequency less than or equal to 0.1% in the Genome Aggregation Database¹⁰ with novel variants absent from gnomAD¹¹ (accessed January 5, 2020). If any novel variants were found more than once in our cohort, we only considered variants with a population frequency of less than or equal to 1.0%. Disease-causing variants fulfilled the pathogenic or likely pathogenic standardized criteria proposed by the American College of Medical Genetics and Genomics, the Association for Molecular Pathology, and the Association for Clinical Genomic Science.^{12,13} Variants were reported on canonical transcripts based on the Ensembl gene tree analysis, and cardiac transcripts were selected based on the highest-expressed isoform in atrial appendage tissue from genotype-tissue expression (GTEx; accessed January 5, 2020).¹¹ All variants under question were required to reside in the exonic regions for both canonical and chosen cardiac transcripts; otherwise, they were excluded from further evaluation.

Sequencing

Blood samples from participants who consented were drawn into 1 vial. The buffy coat layer containing the white blood cells for DNA extraction was collected from the serum. DNA was extracted using a commercially available kit (Gentra Puregene; Qiagen) and samples were stored in a -80 °C freezer. Sequencing was performed on 60 candidate AF genes that have been implicated in mediating AF susceptibility. A custom panel was designed to target all exons from the listed 60 genes (Accel-Amplicon Custom Panel; Swift Biosciences) and was developed in cooperation with the Swift Biosciences team to maximize exon recovery from the genes. The final design consisted of a total of 2449 amplicons, targeting a total of 385 451 base

pairs. Sets of 96 samples were pooled for sequencing (Next-Seq500 instrument; Illumina). The average depth of sequence was $\times 1000$ and on-target rate of aligned reads was 90%, with coverage uniformity greater than 96%. Demultiplexing of sequence data was performed in the cloud storage environment in Basespace for bioinformatics analysis.

Bioinformatics

Overlapping read lengths were merged into single-end reads using paired-end read merger to avoid overcounting variants and provide a higher stringency for more accurate variant calling.¹⁴ We used Trimmomatic to trim reads to ensure that sequences were mapped to the human genome with amplicon primers retained in the reads to improve mapping quality.¹⁵ The trimmed reads were mapped to human reference genome hg19 using Burrows-Wheeler alignment maximal exact match, and any alignments that did not match the amplicon region were removed.¹⁶ We used Genome Analysis Toolkit (GATK) for realignments around indel regions and GATK HaplotypeCaller to identify variants in the candidate genes.¹⁷ The functional annotation of variants was carried out using ANNOVAR.¹⁸

Filtering

We used a stepwise filter approach for identifying rare and novel variants in our multiethnic population. First, standardized quality-control thresholds were required to be met with adequate read depths ($\geq \times 20$), appropriate allelic ratios for heterozygous calls ($\geq 20\%$) with homozygous alternate calls ($\geq 99\%$), and homozygous reference calls completely excluded. Genotype quality scores were not available. If reported read depth values did not closely match ($>10\%$ difference) with manually calculated read depth values, then these variants were considered uninformative reads and not considered any further. Second, we applied a minor allele frequency less than or equal to 0.1% for rare variants and absent for novel variants from the reference gnomAD database. We used gnomAD (versions 2 and 3) as a reference population for the variants we identified, with almost all of them not previously reported in versions 2 and 3 of gnomAD, indicating that they are novel and not identified in a healthy ethnic-specific population. This was only one of the criteria we used to interpret variants. Third, a filtering strategy focused on identifying missense, stop-gain, stop-loss, frameshift, splice-site, and indel coding variants within the exon regions. Fourth, we used cutoff values from in-silico prediction tools for Genomic Evolutionary Rate Profiling (GERP) greater than or equal to 3.0, Sorting Intolerant from Tolerant (SIFT) less than or equal to 0.05, PolyPhen2 greater than or equal to 0.9, MutationTaster greater than or equal to 0.5, protein variation effect analyzer (PROVEAN) less than or equal to -2.5 , and Combined Annotation Dependent Depletion (CADD) greater than or equal to 20. If any variants with more than 1 transcript resulted in multiple in silico scores, we chose the least deleterious value reported to base the imputation. If in silico scores were missing values, we kept these variants because this may have filtered out indels. All variants had a minimum $\times 20$ read depth with allelic balances greater than or equal to 20% for heterozygous

and less than or equal to 1% for homozygous between reference and alternative allele differences to be considered an adequate variant call. Fifth, variants were then classified into pathogenic, likely pathogenic, of unknown significance, likely benign, or benign based on American College of Medical Genetics and Genomics, Association for Molecular Pathology, and Association for Clinical Genomic Science criteria for genes with any prior genetic data suggesting a potential link to AF.^{12,13}

Genotyping

All identified rare and novel variants meeting our stringent threshold underwent Sanger sequencing to confirm variant calls from probands as well as enrolled first-degree family members to assess for cosegregation with AF.

The data were analyzed using SAS, version 9.4 (SAS Institute Inc). For continuous variables, distributed variables are reported as means (interquartile range) using an unpaired *t* test. For categorical variables, frequencies were compared using the Pearson χ^2 test and are reported as count (percentage). When comparing multiple groups, analysis of variance and Mann-Whitney and Kruskal-Wallis tests were used for analysis. A post hoc pairwise comparison was applied with Bonferroni correction. For rare variants, comparisons across subpopulations were performed using the Fisher exact test (1-sided for testing enrichment and 2-sided for testing differences). The threshold for *P* value statistical significance across all analyses was $P \leq .05$.

Results

Clinical Characteristics

The study cohort consisted of 227 probands with early-onset AF; of these, 148 (65.2%) were African American, 79 (34.8%) were Hispanic/Latinx, and 132 (58.1%) were men (Table 1). The mean (SD) age at AF onset was 51.0 (9.9) years. There was a verified family history of AF in 24 probands (10.6%) in the cohort, and we found that 56 (24.7%) of the 227 probands were diagnosed with early-onset AF before developing comorbidities. In addition, African American participants were more likely to be obese and have a history of hypertension, coronary artery disease, and congestive heart failure compared with Hispanic/Latinx participants. eTable 1 in the Supplement reports the comparison of the AF-associated comorbidities in the European American, African American, and Hispanic/Latinx cohorts of early-onset AF probands. We found that the multiethnic cohort had fewer men, younger age at onset for AF, reduced reported family history of AF, and a greater burden of cardiovascular comorbidities (obstructive sleep apnea, hyperthyroidism, coronary artery disease, valvular heart disease, congestive heart failure, hypertension, type 2 diabetes, and stroke) compared with European American individuals.

We reviewed all 227 echocardiograms for the early-onset AF cohort and found that 25 patients (11.0%) displayed a moderate to severe dilated cardiomyopathy (DCM) phenotype. However, only UIC-0358 (*TTN*-107050G>T; OMIM: 188840) proband carried a pathogenic or likely pathogenic variant and had DCM. We also noted that the remaining patients with

Table 1. Baseline Clinical Characteristics of African American, European American, and Hispanic/Latinx Probands With Early-Onset Atrial Fibrillation

Category	No. (%)			P value
	Total (n = 227)	African American (n = 148)	Hispanic/Latinx (n = 79)	
Men	132 (58.1)	83 (56.1)	49 (62.0)	.24
Age at onset, mean (SD), y	51.0 (9.9)	51.7 (9.4)	49.8 (10.8)	.16
Atrial fibrillation				
Paroxysmal	174 (76.7)	118 (79.7)	56 (70.9)	.09
Persistent	37 (16.3)	22 (14.9)	15 (19.0)	.27
Permanent	16 (7.0)	8 (5.4)	8 (10.1)	.15
Family history	24 (10.6)	13 (8.8)	11 (13.9)	.16
BMI, mean (SD)	34.2 (9.3)	35.4 (9.8)	31.8 (7.6)	<.001
Obstructive sleep apnea	42 (18.5)	27 (18.2)	15 (19.0)	.51
Hyperthyroidism	10 (4.4)	7 (4.7)	3 (3.8)	.52
Coronary artery disease	109 (48.0)	79 (53.4)	30 (38.0)	.02
Valve heart disease	51 (22.5)	29 (19.6)	22 (27.8)	.11
Mitral (≥moderate-severe)	36 (15.9)	19 (12.8)	17 (21.5)	.21
Aortic (≥moderate-severe)	9 (4.0)	1 (0.7)	8 (10.1)	<.001
Congestive heart failure	89 (39.2)	68 (45.9)	21 (26.6)	<.001
Hypertension	154 (67.8)	116 (78.4)	38 (48.1)	<.001
Type 2 diabetes	68 (30.0)	49 (33.1)	19 (24.1)	.10
Stroke	28 (12.3)	20 (13.5)	8 (10.1)	.30
Vascular disease	14 (6.2)	12 (8.1)	2 (2.5)	.08
CHA ₂ DS ₂ -VASc score ≥2	123 (54.2)	58 (39.2)	33 (41.8)	.41
Left ventricular hypertrophy, mean (SD), g/m ²	64 (28.2)	41 (27.7)	23 (29.1)	.88
Intraventricular septum, mean (SD), cm	1.0 (0.2)	1.1 (0.2)	1.0 (0.2)	<.001
Relative wall thickness, mean (SD), cm	0.42 (0.1)	0.43 (0.1)	0.42 (0.1)	.47
LVEF, mean (SD), %	81 (35.7)	52 (35.1)	29 (36.7)	.88
Left atrial diameter, mean (SD), mm	4.3 (0.8)	4.3 (0.8)	4.3 (0.9)	.80

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CHA₂DS₂-VASc, congestive heart failure, hypertension, age, diabetes, stroke, vascular disease, and sex; LVEF, left ventricular ejection fraction.

reduced left ventricular ejection fraction had a tachycardia-induced cardiomyopathy related to rapid AF.

Variants

Sequencing 60 candidate genes identified 53 variants. We identified 23 rare variants, but no probands with early-onset AF harbored a disease-causing variant. All variants were classified as variants of unknown significance. We sequenced the coding regions of 60 candidate genes and identified likely pathogenic (43.8%) or pathogenic (56.2%) variants in 16 probands (7.0%) of African and Hispanic descent with early-onset AF.

We identified 30 novel variants, and 17 of these variants were classified as likely pathogenic or pathogenic across 16 probands. In 16 probands with early-onset AF (7.0%), 11 (7.4%) were noted in African American individuals and 5 (6.3%) were observed in Hispanic/Latinx individuals; the remaining 13 variants were classified as variants of unknown significance (Table 2). Six variants (40.0%) encoded for cardiac ion channel proteins, and 3 of them were specific for the cardiac sodium channel gene (*SCN5A*; OMIM: 600163), 1 L-type calcium channel gene (*CACNA1C*; OMIM: 114205), and 2 for the potassium channel genes (*KCNQ1*; OMIM: 607542 and *KCNJ11*; OMIM: 600937). The most common type of variants were mis-

sense (40.0%) and stop-gain (40.0%), followed by frameshift (20.0%). The mean (SD) in silico scores were as follows: GERP++ NR, 5.2 (0.7); GERP++ RS, 4.9 (0.83); SIFT, 0.00 (0.00); Polyphen2 HDIV, 0.99 (0.01); Polyphen2 HVAR, 1.0 (0.01); MutationTaster, 1.0 (0.0); PROVEAN, -4.8 (1.5); and CADD, 39.9 (17.37) (eTable 2 in the Supplement). Comparing the burden of rare variants in the multiethnic subpopulations demonstrated that the reference cohort had a significantly higher number of rare variants than the case cohort ($P \leq .001$). This significant finding also held true when we compared the different subpopulations (African American, 1326 rare variants from a total of 6464 variants; $P \leq .001$ and Hispanic/Latinx, 1456 rare variants from a total of 6517 variants $P \leq .001$) within the reference and case cohorts. Although a low prevalence of rare variants may be related to a cohort enriched with acquired causes of AF, eTable 3 in the Supplement notes that early-onset AF probands with obesity, coronary artery disease, congestive heart failure, and hypertension had a greater prevalence of rare variants.

We identified 8 probands (3.5%) with *TTN* variants encoding the sarcomeric protein titin. Because *TTN* truncating variants are a recognized cause of AF⁷ and not just DCM, we noted that 25 (11.0%) early-onset AF probands manifest

Table 2. Identified Rare and Novel Variants in African American and Hispanic/Latinx Probands With Early-Onset Atrial Fibrillation

Study ID	Race	Chr	Position	Gene	MAF	Transcript ^a	Exon	Nucleotide	Protein	Variation	PSI	Region	Classification
UIC-0066	H/L	2	TTN	179644802	00.00	ENST00000589042	22/363	3654T>A	Y1218 ^a	Stop gain	100	None	LP (PV51, PM2)
UIC-0357	AA	2	TTN	179644802	00.00	ENST00000589042	22/363	3654T>A	Y1218 ^a	Stop gain	100	None	LP (PV51, PM2)
UIC-0019	H/L	2	TTN	179397682	00.00	ENST00000589042	358/363	103660G>T	E34554 ^a	Stop gain	100	M-band	LP (PV51, PM2)
UIC-0160	H/L	2	TTN	179476320	00.00	ENST00000589042	269/363	50636delA	N16879fs	Frameshift	100	A-band	LP (PV51, PM2)
VA-0090	AA	2	TTN	179476320	00.00	ENST00000589042	269/363	50636delA	N16879fs	Frameshift	100	A-band	LP (PV51, PM2)
UIC-0119	AA	2	TTN	179460243	00.00	ENST00000589042	295/363	57838G>T	E19280 ^a	Stop gain	100	A-band	LP (PV51, PM2)
VA-0009	AA	2	TTN	179396890	00.00	ENST00000589042	358/363	104452G>T	E34818 ^a	Stop gain	100	M-band	LP (PV51, PM2)
UIC-0358	AA	2	TTN	179393428	00.00	ENST00000589042	360/363	107050G>T	G35684 ^a	Stop gain	100	M-band	LP (PV51, PM2)
UIC-0134	AA	3	SCN5A	38627224	00.00	ENST000000413689	16/28	2745C>A	C915 ^a	Stop gain	NA	Extracellular	LP (PV51, PM2)
VA-0070	AA	3	SCN5A	38601648	00.00	ENST000000413689	23/28	4235T>G	L1412R	Missense	NA	Pore	LP (PM1, PM2, PP3, BP1)
UIC-0093	H/L	3	SCN5A	38598726	00.00	ENST000000413689	24/28	4295G>T	R1432M	Missense	NA	Extracellular	LP (PM1, PM2, PP3, PM5, BP1)
UIC-0384	AA	4	PITX2	111542364	00.00	ENST00000306732	2/3	367G>C	E123Q	Missense	NA	Homeobox	LP (PM1, PM2, PP3, BP1)
UIC-0056	AA	4	PITX2	111542357	00.00	ENST00000306732	2/3	374T>A	I125N	Missense	NA	Homeobox	LP (PM1, PM2, PP3, BP1)
UIC-0639	AA	4	PITX2	111542357	00.00	ENST00000306732	2/3	374T>A	I125N	Missense	NA	Homeobox	LP (PM1, PM2, PP3, BP1)
UIC-0104	H/L	11	KCNJ11	17408936	00.00	ENST00000339994	1/1	703C>A	Q235K	Missense	NA	Cytoplasmic	LP (PM1, PM2, PP3)
UIC-0407	AA	11	KCNQ1	2604665	00.00	ENST00000155840	7/16	922G>T	V308F	Missense	NA	Pore	LP (PM1, PM2, PP3)
UIC-0104	H/L	12	CACNA1C	2794922	00.00	ENST00000347598	46/49	5741delA	N1914fs	Frameshift	NA	Cytoplasmic	LP (PV51, PM2)

Abbreviations: AA, African American; Chr, chromosome; H/L, Hispanic/Latinx; LP, likely pathogenic; MAF, minor allele frequency; NA, not available; PSI, percent spliced-in.
^a Canonical transcript based on Genome Aggregation Database (gnomAD; accessed January 5, 2020).

Table 3. Clinical Characteristics of Familial AF Kindreds Harboring Rare and Novel Gene Variants of Uncertain Significance

Study ID	Gene variant	Sex	Symptoms	Age at diagnosis, y	AF type	Comorbidities	Treatment	PR interval, ms	QRS duration, ms	LA size, mm	LVEF, %
AA 0174	KCNE5 (R85H)	II:1	Palpitations	48	Paroxysmal	Hypertension, obstructive apnea, obesity	Rhythm	162	82	4.0	60
		II:2	Dyspnea	54	Persistent	Hypertension	Rhythm	148	96	3.8	60
		II:4	Fatigue	59	Permanent	None	Rate	178	102	4.4	60
H/L 0207	NPPA (D36Y)	II:2	Tiredness	58	Paroxysmal	Stroke, pacemaker	Rate	142	104	3.5	60
		II:3	Dyspnea	65	Paroxysmal	Stroke	Rate	138	92	3.0	55
		III:1	Palpitations	41	Paroxysmal	None	Rhythm	158	108	3.8	60
AA 0281	SCN10A (R1142C)	I:2	Palpitations	58	Paroxysmal	Stroke	Rate	134	88	3.5	60
		II:2	Palpitations	61	Paroxysmal	Hypertension	Rhythm (pulmonary vein isolation)	138	90	3.8	55
		III:2	Dyspnea	42	Persistent	Hypothyroidism	Rate	145	110	3.9	60
AA 0473	MYH6 (E1323V)	II:2	Fatigue	59	Paroxysmal	Hypertension, chronic kidney disease	Rhythm	127	72	3.4	50
		II:3	Dyspnea	56	Paroxysmal	Hypertension	Rhythm	142	78	3.6	50
		II:6	Palpitations	61	Persistent	Hypertension	Rate		88	4.6	45
H/L 0526	MYH6 (K1307M)	II:2	Palpitations	62	Paroxysmal	Type 2 diabetes	Rate	154	88	4.4	60
		II:3	Fatigue	58	Persistent	None	Rate	NA	104	NA	55
		II:6	Palpitations	61	Permanent	None	NA	148	94	NA	60
H/L 0606	TTN (G25630V)	I:2	Deceased	68	Permanent	Stroke	Rate control				
		II:2	Palpitations	48	Paroxysmal	None	Rhythm (pulmonary vein isolation)	122	63	3.5	60
		III:1	Palpitations			None		98	98	3.8	60

Abbreviations: AF, atrial fibrillation; LA, left atrium; LVEF, left ventricular ejection fraction; NA, not applicable.

a DCM phenotype by echocardiography (Table 1). However, only UIC-0358 (*TTN*-107050 G>T) proband carried a likely pathogenic or pathogenic variant and had DCM. We also noted that the remaining patients with reduced left ventricular ejection fraction developed a tachycardia-induced cardiomyopathy associated with rapid ventricular rates during AF. We established a diagnosis of AF-related DCM by reviewing echocardiograms performed before and after treatment of early-onset AF.

Cosegregating Variants

None of the early-onset AF probands carrying a disease-causing variant reported a family history of AF. However, in 6 probands (2.6%) with more than 2 affected family members,

variants of unknown significance were identified that cosegregated with AF (Table 3). We identified 3 rare variants in genes encoding sodium channel protein type 10 subunit α (*SCN10A*; OMIM: 604427), voltage-gated potassium channel accessory subunit 5 (*KCNE5*; OMIM: 300328), and titin (*TTN*), and 3 novel variants in myosin heavy chain 6 (*MYH6*; OMIM: 160710 [2 variants]) and natriuretic peptide precursor A (*NPPA*; OMIM: 108780) that cosegregated with AF (Figure 1 and Figure 2). The mean (SD) read depth for these variants was 509.5 (678.2). The mean (SD) for the novel variants was 102.2 (125.2) and mean (SD) in silico scores were as follows: GERP++ NR, 5.0 (0.7); GERP++ RS, 4.9 (1.0); SIFT, 0.0 (0.0); Polyphen2 HDIV, 1.0 (0.0); Polyphen2 HVAR, 1.0 (0.01); MutationTaster, 1.0 (0.0); PROVEAN, -5.8 (1.4); and CADD, 28.1 (2.7) (eTable 3 in the Supplement).

Figure 1. Pedigrees of 3 Families Carrying Rare Candidate Atrial Fibrillation (AF) Gene Variants

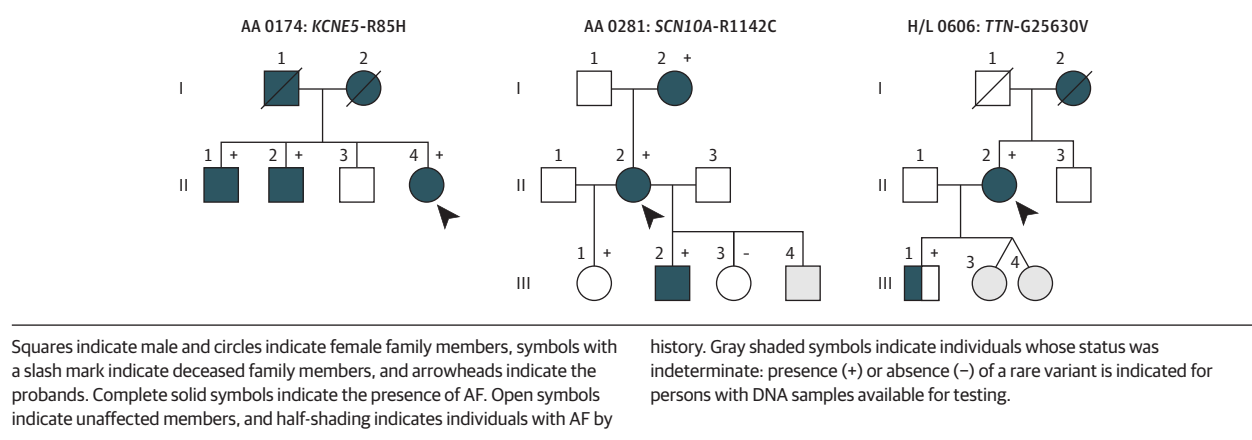
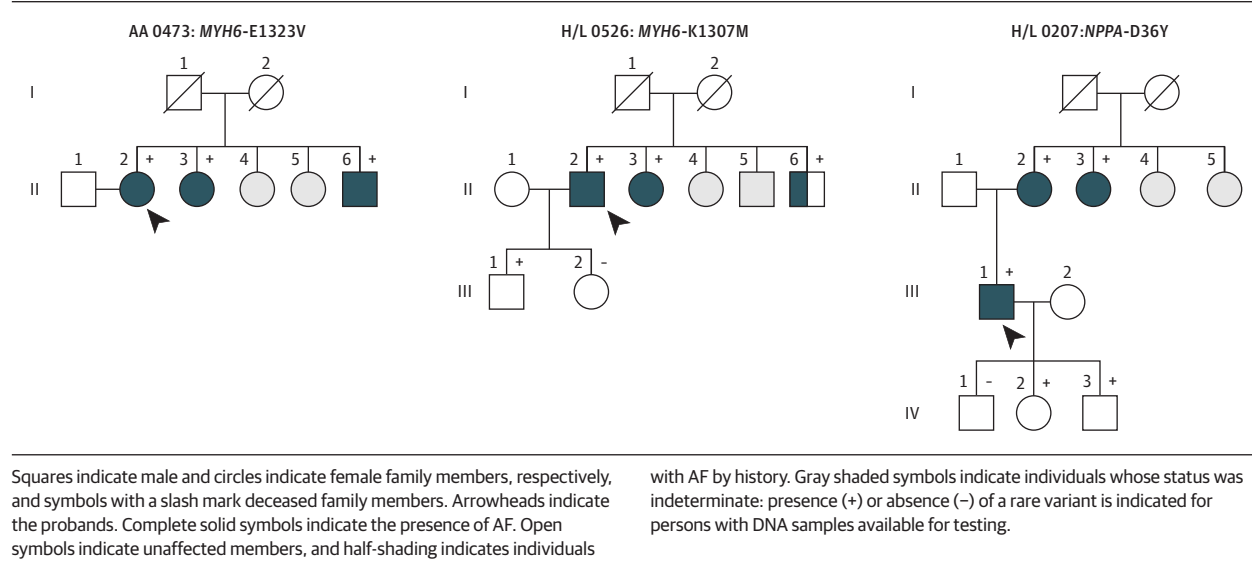


Figure 2. Pedigrees of 3 Families Carrying Novel Candidate Atrial Fibrillation (AF) Gene Variants



Discussion

We sequenced the coding regions of 60 candidate genes and identified likely pathogenic (43.8%) or pathogenic (56.2%) variants in 16 probands (7.0%) of African and Hispanic descent with early-onset AF, with most (46.7%) loss-of-function variants in the *TTN* gene. In addition, 6 novel and rare variants encoding cardiac sodium (*SCN10A*) and potassium channels (*KCNE5*), myocardial structural proteins (*MYH6* and *TTN*), and signaling molecules (*NPPA*) cosegregated with AF in multiple affected family members. Although most ethnic minority probands with early-onset AF did not harbor rare variants, our findings not only represent progress toward the molecular phenotyping of AF but also identify novel mechanism-based therapeutic approaches for this common arrhythmia in ethnic minority individuals.

We identified rare variants associated with AF in a small percentage (7.0%) of ethnic minority probands with early-

onset AF. This percentage is lower than that found in European White individuals with early-onset AF, of whom approximately 20% carried very rare variants.¹⁹ There are several potential explanations for why a minority of probands of self-reported African American and Hispanic/Latinx ethnicity carried rare gene variants. Because our ethnic cohort, especially African American individuals, had a greater burden of AF-associated comorbidities, one reason may relate to a population enriched with acquired causes. Our probands did not have lone AF. However, we noted that that probands with early-onset AF with comorbidities had a greater prevalence of rare variants, suggesting that these may be risk alleles rather than mendelian disease-associated variants (eTable 3 in the Supplement). Nonetheless, a study across race/ethnicity populations supports the concept of the 2-hit hypothesis in which susceptibility to AF is dependent not only on carrying a pathogenic variant associated with AF, but also on a second hit, such as an established AF risk factor (eg, obesity or hypertension) or common genetic variants on chromosome 4q25.²⁰ Other

potential explanations for why a minority of African American and Hispanic/Latinx probands with early-onset AF harbored rare variants include the failure to sequence genes not yet identified that are involved in the pathogenesis of early-onset AF; sequencing only the coding regions of candidate genes; rare variants with a minor allele frequency less than or equal to 0.1% and missing variants or combinations of variants with intermediate effects; and the challenges of correctly phenotyping participants with AF, which can be asymptomatic.

We identified 8 probands (3.5%) of African and Hispanic descent with *TTN* variants encoding the sarcomeric protein titin. This prevalence may in part reflect a higher burden of cardiovascular risk factors, especially in African American individuals, creating a substrate for atrial myopathy and re-entrant AF.²¹ Titin acts as a molecular scaffold for sarcomeric assembly and directly interacts with actin and myosin filaments to provide stability during the contraction and relaxation phases of the cardiac cycle.²² Variants in *TTN* have been associated with dilated and hypertrophic cardiomyopathy, neuromuscular disorders, and, most recently, early-onset AF.^{7,23-25} Thus, another possible explanation for the high frequency of *TTN* variants in the early-onset AF cohort in the present study is that AF is associated with DCM. However, echocardiographic characterization of the probands carrying *TTN* revealed that 11.0% of the cohort had evidence of DCM and only 1 kindred (UIC-0358) harbored a likely pathogenic or pathogenic variant (Table 2). UIC-0358 proband is better classified as a *TTN* cardiomyopathy because there was evidence of DCM by echocardiography. However, at this time, UIC-0357 proband, who also carries a pathogenic or likely pathogenic variant, is not classified as a *TTN* cardiomyopathy because there is no evidence of DCM. The underlying mechanisms by which these variants cause AF remain unclear, and further research directed at functionally characterizing *TTN* loss-of-function variants in vitro and in vivo is required. An improved understanding of the pathophysiologic mechanisms by which *TTN* variants cause AF will also enable a more mechanism-based approach to AF therapy in African American and Hispanic/Latinx individuals with early-onset AF because current antiarrhythmic drug therapy targets cardiac ion channels. We also identified 2 new variants in *MYH6* that cosegregated with AF in multiple family members, further supporting the association between myocardial integrity and the development of AF.²⁶⁻²⁹

Our findings have several potential clinical implications. First, an improved understanding of the underlying genetic mechanisms by which pathogenic variations, especially those in *TTN*, cause AF may identify a new therapeutic target for the arrhythmia across race/ethnicity because current antiarrhythmic therapy is limited and directed at cardiac ion channels. Second, our findings have potential implications for the screening and assessment of African American and Hispanic/Latinx individuals with early-onset AF. Cascade screening will identify family members at increased risk for developing AF. Third, although gene sequencing identified pathogenic variants in only a small percentage of participants, there was a high prevalence of *TTN* variants in probands of self-reported African

American and Hispanic/Latinx ethnicity. However, studies focused on determining the utility of sequencing candidate genes encoding myocardial structural proteins like *TTN* are needed, especially in individuals with AF onset before age 40 years.³⁰

Limitations

This study has some limitations. First, there is great diversity in Hispanic/Latinx ancestry, especially in gnomAD. However, more than 75% of the Hispanic probands with early-onset AF were of Mexican descent, as previously described.⁹ Race/ethnicity was self-reported rather than determined by genetic ancestry. Second, we did not perform linkage analysis and quantify cosegregation with informative meiosis because of the limited number of multiethnic kindreds. However, the likelihood of misclassifying variants was low given the stringent criteria we used to classify them. Third, we sequenced 60 candidate genes implicated in the pathogenesis of AF. Despite this extensive target panel of genes, additional candidate genes remain unknown. Fourth, even though we sequenced the coding region of most of the cardiac ion channel proteins, signaling molecules, and transcription factors, the calcium auxiliary subunit channel genes or an expanded list of myocardial structural protein genes were not screened owing to size restriction in the gene panel. This lack of screening may underestimate the true prevalence of pathogenic variants seen in individuals of ethnic minority with early-onset AF. Fifth, despite good overall coverage across 60 candidate genes, approximately 4% per gene was uncovered in the exonic regions. It is thus possible that we missed a small number of variants residing in these challenging regions. Sixth, genome-wide association studies have identified a large number of AF loci that reside in noncoding regions. Although our study did not assess common genetic variation as an etiologic factor associated with early-onset AF in individuals of ethnic minority, the chromosome 4q25 locus has been associated with increased susceptibility to AF in Hispanic/Latinx individuals.⁹ Seventh, our filtering threshold used a minor allele frequency less than or equal to 0.1% to identify rare variants. We did not assess the frequency of less rare variants or combinations of variants in the cause of early-onset AF in African American and Hispanic/Latinx individuals. Eighth, formal gene to disease associations were not curated in a stringent way, and future studies will need to do this to determine which genes should be considered for genetic testing in clinical practice when applying these diagnostic criteria.

Conclusions

To our knowledge, this is one of the first studies to sequence candidate genes in ethnic minority probands with early-onset AF. We identified likely pathogenic and pathogenic variants with most loss-of-function variants in *TTN* that increase susceptibility to AF in African American and Hispanic/Latinx individuals. Our findings not only represent progress toward molecular phenotyping of AF but also identify novel mechanism-based therapeutic approaches for AF in ethnic minority populations.

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