ARTICLE

Genetic testing in monogenic early-onset atrial fibrillation

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A substantial proportion of atrial fibrillation (AF) cases cannot be explained by acquired AF risk factors. Limited guidelines exist that support routine genetic testing. We aim to determine the prevalence of likely pathogenic and pathogenic variants from AF genes with robust evidence in a well phenotyped early-onset AF population. We performed whole exome sequencing on 200 early-onset AF patients. Variants from exome sequencing in affected individuals were filtered in a multi-step process, prior to undergoing clinical classification using current ACMG/AMP guidelines. 200 AF individuals were recruited from St. Paul's Hospital and London Health Sciences Centre who were ≤ 60 years of age and without any acquired AF risk factors at the time of AF diagnosis. 94 of these AF individuals had very early-onset AF (≤ 45). Mean age of AF onset was 43.6 ± 9.4 years, 167 (83.5%) were male and 58 (29.0%) had a confirmed family history. There was a 3.0% diagnostic yield for identifying a likely pathogenic or pathogenic variant across AF genes with robust gene-to-disease association evidence. This study demonstrates the current diagnostic yield for identifying a monogenic cause for AF in a well-phenotyped early-onset AF cohort. Our findings suggest a potential clinical utility for offering different screening and treatment regimens in AF patients with an underlying monogenic defect. However, further work is needed to dissect the additional monogenic and polygenic determinants for patients without a genetic explanation for their AF despite the presence of specific genetic indicators such as young age of onset and/or positive family history.

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INTRODUCTION

Atrial Fibrillation (AF) is the most common sustained cardiac arrhythmia worldwide and is associated with significant morbidity and increased mortality [1, 2]. It is estimated that over 1 in 3 Americans will develop AF in their lifetime [3]. A substantial proportion of AF in this population cannot be explained by recognized acquired AF risk factors [4]. Over the years, considerable advancements have been made to better understand the genetic aspects of AF [5]. Multiple approaches have led to the discovery of common and rare contributing variants in cardiac ion channels, structural proteins and signaling molecules [6]. Several studies have highlighted the importance of utilizing the underlying genetic architecture of an individual patient's arrhythmia to guide therapeutic strategies, but few studies have been able to demonstrate the value of this in the real world of clinical practice [7]. Limited guidelines exist that support routine genetic testing, and current recommendations suggest that, in rare circumstances, patients who develop AF at a young age and have a positive family history or additional features suspicious of other forms of inherited cardiac disease, may undergo genetic testing to identify a potential causative factor [8, 9]. To a large degree, it remains uncertain which genes to test, whether genetic testing would be of benefit in a clinical setting, and precisely how this would alter care for these patients. Our goal for this study was to elicit the prevalence of likely pathogenic and pathogenic variants present in AF genes with robust evidence that are clinically actionable in a well phenotyped early-onset AF population. This approach would not only define the prevalence of disease-causing variants for a monogenic cause in AF, but also provide insight into the potential clinical utility for offering different screening and treatment regimens in AF patients with an underlying monogenic defect.

METHODS

Definitions

Very-early-onset AF was defined as AF with onset ≤ 45 years of age. Early-onset AF was defined as AF > 45, but ≤ 60 years of age. Both groups had complete absence of cardiovascular risk factors or overt heart disease. Familial AF was defined as the presence of AF in ≥ 1 first-degree relatives. The label "disease-causing" was given to variants classified as pathogenic or likely pathogenic and "non-disease-causing" variants corresponded to likely benign and benign classification, but also included variants of unknown significance. We included and described variants according to the canonical transcript, which was designated as the longest consensus coding sequence from Ensembl. We also reported variants occurring on cardiac transcripts, which were selected based on most highly expressed transcript in the left atrial appendage from Genotype-Tissue Expression (GTEx).

Study population

All 200 unrelated patients were recruited from a multidisciplinary AF clinic at St. Paul's Hospital in Vancouver, Canada and from London Health

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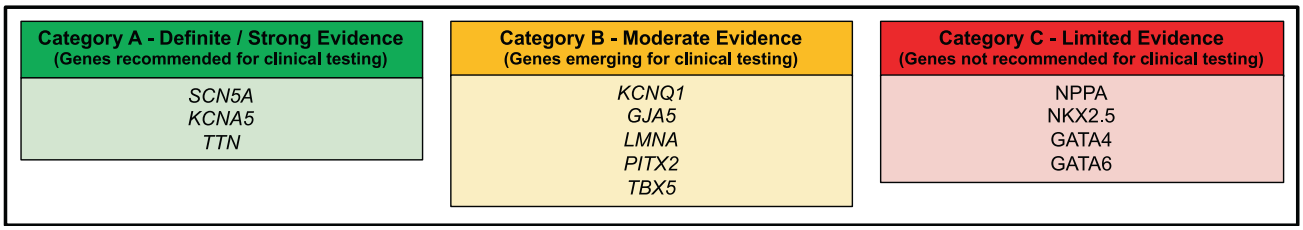

Fig. 1 Categorizing atrial fibrillation genes based on the ClinGen framework for gene to disease association. A list of genes that have been group into definite and strong, moderate, and limited evidence-based categories.

Sciences Centre, London, Canada from July 2017 to August 2019. A 12 lead ECG confirming AF diagnosis was required. These were overread by cardiac electrophysiologists (authors ZL). All individuals had an extensive clinical workup with clinical history, physical examination, ECGs, Holter monitoring, and echocardiograms to rule out other cardiovascular risk factors or overt heart diseases such as moderate or severe forms of obesity, hyperthyroidism, obstructive sleep apnea, hypertension, diabetes mellitus, coronary artery disease, valvular heart disease, cardiomyopathy, congestive heart failure, transient ischemic attacks or stroke. While not part of the prospective enrollment, a review of the clinical management and outcomes of enrolled patients revealed that all patients under the age of 30 have undergone electrophysiology studies and none of the study participants were found to have AVRT, AVNRT, or a concealed accessory pathway. Anyone who was ≤ 60 years old with complete absence of cardiovascular risk factors underwent whole exome sequencing.

Gene selection

We curated a list of 12 candidate AF genes from the published literature, which comprised of relevant ion channels, structural proteins, signaling molecules, and transcription factors (*SCN5A, KCNA5, TTN, PITX2, KCNQ1, GJA5, LMNA, TBX5, NPPA, NKX2.5, GATA4, GATA6*). For this list of associated genes, we more deeply mined the literature to determine which genes had sufficiently robust evidence to meet gene-to-disease association criteria as per the Gene-Disease Validity Standard Operating Procedures (version 8) established by ClinGen [10]. We also included an additional list of hypertrophic (*ACTC1*, *FLNC*, *MYH7*, *PLN*, *TNNI3*, *TNNT2*, *TPM1*, *ACTN2*, *CSRP3*, *JPH2*, *TNNC1*) and dilated (*FLNC*, *LMNA*, *MYH7*, *SCN5A*, *TNNC1*, *TNNT2*, *TTN*, *ACTC1*, *JPH2*, *NEXN*, *TNNI3*, *TPM1*, *VCL*) cardiomyopathy (CM) genes, which did not require curation since they have already been deemed to have sufficient gene to disease evidence by the ClinGen team [11, 12].

Gene curation

We applied the gene to disease framework established by ClinGen for the selected AF candidate genes: SCN5A, KCNA5, TTN, PITX2, KCNQ1, GJA5, LMNA, TBX5, NPPA, NKX2.5, GATA4, GATA6 (Fig. 1). Given that multiple phenotypes exist for these AF candidate genes, we carefully used the lumping and splitting guidelines set out by ClinGen. This resulted in us splitting each AF candidate gene into its separate disease entities, taking into account phenotypic variability, molecular mechanism, and inheritance pattern. Next, published genetic and experimental data were reviewed and assessed in the literature for these AF candidate genes and a score was given for each publication, which was summed at the end to determine classification: definite and strong evidence (clinically relevant (≥ 12 points)), moderate evidence (borderline clinically relevant (7–11 points)), limited evidence (not currently clinically relevant (1–6 points)) and no evidence (unknown). The maximum amount of points allowed for the genetic evidence section was 12, and maximum amount of points allowed for the experimental evidence category was 6. In the genetic evidence section, scoring was tailored toward the type of study under evaluation. Points were awarded based on the type of deleterious rare variant, presence of convincing functional data, and presence of a significant degree of segregation for case level studies. Points were also awarded for case-control studies if the rare variant analysis was statistically significant. In the experimental evidence section, points were awarded based on the functional alteration and model system used for any given study. It is important to highlight that if there was an individual variant with functional data, we used the genetic evidence section to score this piece of data. However, if functional data was related to the given gene under evaluation, we used the experimental evidence section to score this piece of data. The curation process was performed using the two biocurator methodology system by independent evaluators (BC and AL) with formal clinical genetics training. Disputes between evaluation of genes was determined through a third independent evaluator (ZL) with cardiac electrophysiology and cardiogenetic training.

Sequencing

Genome Quebec's Canadian Centre for Computational Genomics performed exome sequencing on the Illumina NovaSeq 6000 sequencer using Roche Nimblegen SeqCap EZ Human Exome capture. Raw exome sequencing data was trimmed and aligned to reference human genome 19 (hg19) using BWA MEM [13–15]. Refinements of mismatches near indels were performed using GATK indels realignment to improve read quality post alignment [16]. Variants from the processed reads were identified using either samtools mpileup or GATK haplotype callers. The Genome in a Bottle dataset was used to select steps and parameters minimizing the false positive rate and maximizing the true positive variants to achieve a sensitivity of 99.7%, precision of 99.1%, and F1-score of 99.4% [17].

Filtering

The average gene coverage ≥ 10X across robust AF genes (*SCN5A, KCNA5, TTN, PITX2, KCNQ1, GJA5, LMNA, TBX5*) ranged from 85.7% to 99.9%. Only KCNQ1 and KCNA5 resulted in average coverage below ≤ 99%. *KCNQ1* was found to have an average coverage of 85.7%, but after review of individual exons, exon 1 was found to be poorly covered (26.6%), whereas all other exons were ≥ 99%. The average coverage for *KCNA5* was 98.0%. The average percentage of reads with Q > = 20 and Q > = 30 were 99.9% and 94.9% respectively. We only considered variant calls with read depths ≥ 20X, allelic balance ≤ 20% and ≥ 80%, and genotype qualities ≥ 99% for all reported variants across the AF and CM genes. A filter was applied for variants residing in the exonic regions and resulting in missense, stopgain, stoploss, indel, splice acceptor, or splice donor alterations. All variants were required to have a minor allele frequency (MAF) ≤ 1.0% in gnomAD across all subpopulation frequencies and predicted to be well conserved with damaging effects on protein function scores (GERP NS/NR + + ≥ 3, SIFT ≤ 0.05, PolyPhen2 HDIV/HVAR ≥ 0.90, MutationTaster ≥ 0.50, CADD (PHRED) ≥ 20). There were no canonical splice site (+/−1 or 2 base positions) variants that met our initial thresholds and therefore we did not use further prediction tools with GeneSplicer, MaxEntScan, NNSplice or PWM (Fig. 2).

Classification

Only variants identified in genes with robust evidence for causing AF underwent variant classification. This was also the case for the CM genes. Variants were initially analyzed using VarSeq and VSClinical software (Golden Helix, Bozeman, MT, USA). Any variants meeting our thresholds further underwent manual evaluation by 2 independent assessors with experience with variant assignment. Any discrepancies in interpretation were discussed within our team to reach a general consensus. We classified variants based on the canonical transcript into pathogenic, likely pathogenic, variants of unknown significance, likely benign, or benign using the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) guidelines [18]. We also used Association for Clinical Genomic Science (ACGS) guidelines to further support the above guidelines. Some of these existing criteria contain elements open to user interpretation, and so we devised supplemental criteria appropriate for monogenic AF (Fig. 3).

Statistical analysis

We used an unpaired Students' t-test for continuous variables and a one-tailed Fisher Exact test for categorical variables. The threshold for p-value statistical significance was ≤0.05.

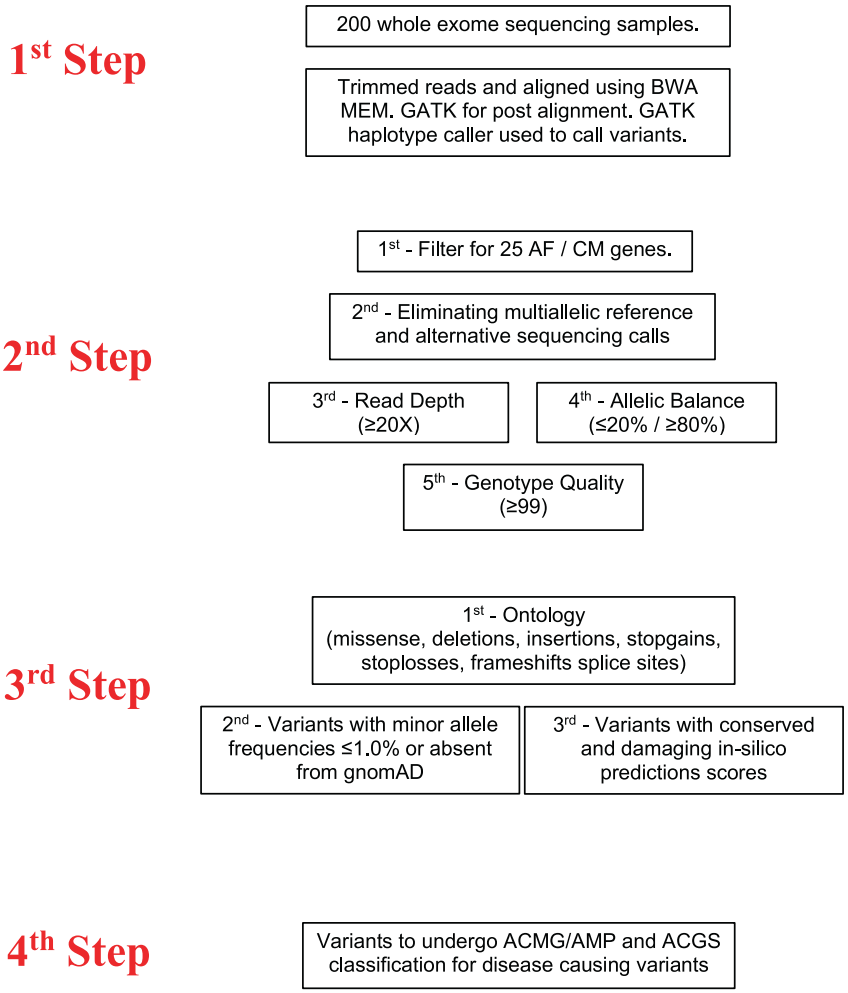

Fig. 2 Flow diagram used to identify variants across atrial fibrillation genes with robust evidence. A flow diagram showing the custom steps taken to filter variants accordingly prior to undergoing variant classification. AF Atrial Fibrillation, CM Cardiomyopathy, ACMG American College of Medical Genetics and Genomics, AMP Association for Molecular Pathology, ACGS Association of Clinical Genomic Science.

RESULTS

Clinical phenotype

There were 200 early-onset AF patients who underwent exome sequencing. Complete clinical data were available for 94 patients with very early-onset AF and 101 patients with early-onset AF. There were 17 patients for whom age or critical clinical details were not available and therefore were not included in the baseline result tables. The primary ethnicity was European (82.1%) with fewer reporting Asian (15.3%) or First Nations (2.6%) ethnicity. The mean age of AF onset was 43.6 ± 9.4 years, 167 (83.5%) were male, and 58 (29.0%) had a confirmed family history. When we stratified our patient population into very early-onset AF and early-onset AF, there was no statistical significance seen for sex or type of AF (persistent or permanent). However, we did find that paroxysmal AF was statistically significant in the very early-onset AF group ($P = 0.04$). Statistical significance was almost met for greater likelihood of a positive family history in very early-onset AF patients ($P = 0.056$). Overall, this was a relatively healthy cohort of patients with AF, and no patients had any self-reported or documented cardiac co-morbidities prior to AF development (Table 1).

Genetic interpretation

Using the gene to disease framework established by ClinGen, we deemed 3 AF genes (*SCN5A, KCNA5, TTN*) to have definite or strong evidence, 5 genes (*KCNQ1, GJA5, LMNA, PITX2, TBX5*) with moderate evidence, and 4 genes (*NPPA, NKX2.5, GATA4, GATA6*) with limited evidence based on the current literature. Despite *PITX2* having enough literature evidence to be classified into the definite / strong evidence category, it was recommended amongst the authors that it be kept within the moderate evidence category. The AF genes that were deemed to have robust evidence (*SCN5A, KCNA5, TTN, KCNQ1, GJA5, LMNA, PITX2, TBX5*), underwent a primary genetic evaluation. A secondary genetic evaluation was carried out for the definite, strong, and moderate evidence hypertrophic (*ACTC1*, *FLNC*, *MYH7*, *PLN*, *TNNI3*, *TNNT2*, *TPM1*, *ACTN2*, *CSRP3*, *JPH2*, *TNNC1*) and dilated (*FLNC*, *LMNA*, *MYH7*, *SCN5A*, *TNNC1*, *TNNT2*, *TTN*, *ACTC1*, *JPH2*, *NEXN*, *TNNI3*, *TPM1*, *VCL*) CM genes. We clinically evaluated all variants that passed our stringent informatic and filtering thresholds for determining pathogenicity. There were 6 (3.0%) AF patients identified to have a variant classifiable as likely pathogenic or pathogenic, all residing in the *KCNQ1* and *TTN* genes (Table 2). The *KCNQ1* variant was a 5-base pair deletion in exon 3 that resulted in a new reading frame and protein truncation. This male individual developed AF at 51 years of age and his family history status was unknown. He had no associated QT abnormalities noted on the electrocardiogram (QT interval was 388 ms and QTc interval was 453 ms). There were 4.3% (4/94) of individuals within the ≤ 45 year old age group and 1.0% (1/101) of individuals within the > 45 year old age group that were identified to have variants in the *TTN* gene. Of these, 3 male individuals with *TTN* variants, developed AF at 41, 31, and 18 years of age and were identified to have a

	ACMG / AMP and ACGS Specific Criteria used for Classifying Variants.
PVS1	Any null variant resulting from stop gain, frameshift, and canonical splice sites outside the last 50 base pairs of the penultimate exon or last exon at the 3-prime end. Null variants must be a recognized cause for disease.
PS1	Any nucleotide change resulting in the same amino acid that has been previously reported as pathogenic or likely pathogenic in the literature.
PS2	We did not use this criterion since maternity or paternity status was not confirmed.
PS3 / BS3	Any validated functional study that was conducted on the specific variant under question and characterized the effect of that variant on protein function.
PS4 / BS1 / BS2	Any variant previously identified in affected individuals with a MAF ≤0.1% or in unaffected individuals with a MAF ≥1.0% from gnomAD.
PM1	Any variant located in a defined critical functional domain. Any variant located in a hot spot region defined as at least 5 pathogenic or likely pathogenic variants reported within a span of 15 base-pairs on either side of the variant under question.
PM2	Any variant that was absent from gnomAD.
PM3 / BP2	We did not use this criteria since parental testing was not done.
PM4 / BP3	Any detectable in-frame deletion or insertion occurring in a nonrepeat region that alters protein length. If any variant resulted in a stoploss, PM4 criteria were used. On the contrary, stopgain variants were assessed under the PVS1 criteria.
PM5	Any nucleotide change that has yet to be reported, but a different nucleotide alteration at the same position is documented as likely pathogenic or pathogenic in the literature.
PM6	We did not use this criterion since de novo rate is not clear in our phenotype.
PP1 / BS4	We did not use this criterion since co-segregation was not confirmed across family members.
PP2 / BP1	Any ratio threshold of ≥50% when comparing pathogenic and likely pathogenic variants against likely benign and benign variants. Missense variant must be a common mechanism of disease.
PP3 / BP4	Any variant that met each in-silico prediction tool threshold: GERP ≥4.0, SIFT ≤0.05, PolyPhen2 HDIV ≥0.90, PolyPhen2 HVAR ≥0.90,MutationTaster ≥0.5 and CADD PHRED ≥20.0.
PP4	We did not use this criterion since it is not appropriate for conditions with significant phenotype heterogeneity.
PP5 / BP6	We did not use these criteria since it is not recommended anymore.

Fig. 3 Specific criteria used to clinically evaluate variants across atrial fibrillation genes that met our filtering criteria. A figure showing an extrapolated list of criteria used to classify variants into pathogenic, likely pathogenic, variant of unknown significance, likely benign and benign categories. ACMG American College of Medical Genetics and Genomics, AMP Association for Molecular Pathology, ACGS Association of Clinical Genomic Science.

nonsense, missense and frameshift variant respectively. All variants had a 100 percent spliced in index and were located in the A-Band of the Ig-Like (R17337*) and Fibronectin Type III domains (W31729C and S21134Wfs*25). The patients who had the nonsense and frameshift *TTN* variants were noted to have a prolonged QTc interval of 454 ms (R17337*) and 492 ms (S21134Wfs*25). The other 2 male individuals had a nonsense and a frameshift *TTN* variant (Q1553* and V3969Lfs*67) with a 100 percent spliced in index and were located near the Z-Disc and in the I-Band. These individuals had a normal QTc interval. Overall, average QTc interval was 420.6 ms (24.9 SD) in our cohort of patients. The average QTc interval across patients < 45 years of age was 417.22 ms (22.0 SD). The average QTc interval across patients > 45 years of age was 423.70 ms (27.1 SD). In addition, there were 2 (1.0%) AF patients who were found to have a variant in a CM gene (*NEXN* and *MYH7*). The patient containing a heterozygous variant (Q265*) in the *NEXN* gene was a male who developed AF at 37 years of age with a family history of AF. There was no evidence of cardiomyopathy on cardiac imaging. This nonsense variant resided in exon 10 and resulted in a premature stop codon and protein truncation (Table 3). The other patient with a heterozygous variant (R1420W) in the *MYH7* gene was male who developed AF at 51 years of age with an unknown family history. There was no evidence of cardiomyopathy on cardiac imaging. This missense variant resides in exon 31 and resulted in an amino acid substitution from arginine to tryptophan (Table 3).

DISCUSSION

This study applies a systematic approach for curating and classifying genes using the ClinGen framework for AF. We highlight 8 genes (*SCN5A*, *KCNA5*, *TTN*, *PITX2*, *KCNQ1*, *GJA5*, *LMNA*, *TBX5*) that contain definite or strong, and moderate literature evidence. We have shown in this study that there is a 3.0% diagnostic yield for identifying a likely pathogenic or pathogenic variant across AF genes with robust evidence from a large cohort of well-phenotyped early-onset AF patients. We also found that there was an additional 1.0% of our patients who had a likely pathogenic or pathogenic variant in a hypertrophic or dilated CM gene. This not only provides new clinical insight and guidance to clinicians for genetic evaluation of AF, but it also highlights the potential clinical utility for offering different screening and treatment regimens in AF patients with an underlying monogenic defect.

This study identified likely pathogenic and pathogenic variants in the *KCNQ1* and *TTN* genes. Previous studies have shown that AF can result from variants affecting *KCNQ1*, as seen in our cohort of patients [19]. Studies identifying variants residing in *KCNQ1* have shown that both gain of function (GOF) and loss of function (LOF) effects can give rise to an AF phenotype. It is thought that the repolarization defects seen for the GOF variants shortens the effective refractory period and thus the action potential duration, but the underlying electrophysiological mechanism for LOF variants are less clear [20]. Other studies have found that variants in *SCN5A*, *KCNA5*, *LMNA*, PITX2, *TBX5*, and *GJA5* can result in AF as

Table 1. Baseline clinical characteristics for our atrial fibrillation patient population.

Category	Very EOAF (18–45yo) ($n = 94$)	EOAF (45–60yo) ($n = 101$)	*P*-Value
Sex (Male)	81 (86.2%)	81 (80.2%)	0.18
Ethnicity			
-European	73 (77.7%)	88 (87.1%)	0.06
-African	0 (0.0%)	0 (0.0%)	0.99
-Asian	17 (18.1%)	12 (11.9%)	0.16
-Hispanic	2 (2.1%)	1 (1.0%)	0.47
-First Nation	2 (2.1%)	0 (0.0%)	0.23
Age at AF Diagnosis	35.73 (± 7.0)	50.71 (±3.37)	< 0.001
Paroxysmal Atrial Fibrillation	86 (91.5%)	83 (82.2%)	0.04
Persistent Atrial Fibrillation	6 (6.4%)	12 (11.8%)	0.14
Permanent Atrial Fibrillation	0 (0.5%)	4 (3.9%)	0.07
Family History	33 (35.1%)	24 (23.5%)	0.056
Body Mass Index	24.3 (± 8.9)	24.6 (±8.5)	0.84

EOAF Early-Onset AF.

well [21–26]. More recently, genetic defects in *TTN* have gained much attention owing to the concept that AF may develop from an atrial myopathy substrate, which highlights the heterogeneity of underlying mechanisms involved in the AF pathogenesis [27–30]. The diagnostic yield for identifying a *TTN* variant appears to be much higher in the ≤ 45 year old group for our study, consistent with prior evidence. Interestingly, one of the *TTN* (W31729C) variants that we identified affects a residue previously reported to cause hereditary myopathy with early respiratory failure (HMERF), but we also suspect it could underlie this subject's AF phenotype, since AF has been reported as part of the HMERF spectra of sequelae [31]. This study also identified likely pathogenic variants in the *MYH7* and *NEXN* genes. Recent studies have shown a high burden of variants residing in the *MYH7* gene from patients with AF [32]. However, only 0.5% of our cohort of patients were identified to have a likely pathogenic or pathogenic variant. We suspect the disparity in the reported *MYH7* variants relates to the fact that we used gene specific guidelines for classifying and interpreting genetic findings in *MYH7* [33]. Our *MYH7* variant (R1420W) had well-established evidence of pathogenicity, but it does not appear that any of these previous studies had a proband with cardiomyopathy and atrial fibrillation. There is less well-established evidence for an association with variants in *NEXN* and AF.

Despite targeting a well-phenotyped population of early-only AF individuals, only a small proportion of AF patients were found to have a likely pathogenic or pathogenic variant in genes with robust evidence that could explain their early AF onset presentation. However, previous studies have highlighted a higher prevalence of likely pathogenic or pathogenic variants across AF genes [32, 34]. The discrepancy between our study and previously published literature can be potentially explained by the following: (1) Less diverse patient population. (2) Strict clinical phenotype for defining our early-onset AF population. (3) Narrow set of genes that only contained robust evidence. (4) Stringent variant filters and interpretations of our genetic findings. Furthermore, it is also important to highlight why patients in our study who had a high suspicion for a genetic cause, nonetheless lacked a genetic explanation. This can be possibly explained by the following: (1) We only curated evidence for 12 genes and further curation efforts are needed to expand from our original list of genes to determine if further genetic screening and evaluation are needed, which may account for some of our patients without a genetic explanation. (2) Any variants that did not meet our filtering criteria were filtered out early on and not assessed with guideline criteria, and it is possible some were likely pathogenic or pathogenic. (3) We identified several patients with a variant of unknown significance, which will require functional assays or other lines of evidence to identify those variants that are truly likely pathogenic or pathogenic. (4) We did not examine variants located in the 3-prime or 5-prime untranslated regions, nor were intronic, intergenic, or large copy number variants assessed. Exploring these regions will certainly provide additional insight and potentially account for other patients with an underlying genetic cause. 5) Finally, our study assumed a monogenic model, and it is likely that at least a portion of this cohort has a polygenic etiology for their early-onset AF status, which we did not explore.

There are currently limited guidelines suggesting routine genetic testing for familial or early-onset AF, and there are limited genetically-driven management strategies being implemented in routine clinical practice. However, other forms of inherited cardiovascular disease have already implemented pharmacogenomic treatment strategies to their guidelines [35, 36]. Several studies to date have identified rare variants in candidate genes for AF, but defining which genes are clinically actionable has been a barrier for implementing routine treatment strategies that target the underlying genetic defect. It is increasingly being recognized that a large number of these individuals have a concurrent arrhythmia or cardiomyopathy phenotype and careful surveillance could be of benefit for early detection for these patients. Ultimately, identifying a likely pathogenic or pathogenic variant in one of the clinically actionable AF genes can have potential strong clinical implications for the future management of AF, which would include the following: (1) Facilitating appropriate screening for other associated phenotypes such as inherited arrhythmias and cardiomyopathies. (2) Selecting a more targeted ant-arrhythmic agent to improve symptom control and reduce the risk for potentially lethal off-target side effects. (3) Guidance toward ablation strategies (pulmonary vein isolation vs. substrate modification) for achieving better success rates. (4) Prognostication of AF course at the time of diagnosis or screening will help guide decision making, such as genetic defects in structural genes. The different clinical management implications related to the genetic underpinnings for AF have been described in a recent publication [37].

In addition to the limitations that have already been mentioned above, this study has a few limitations that should also be acknowledged. (1) Paroxysmal AF was noted to be statistically significant in the very early-onset AF group when compared to the early-onset AF group, which could be highlighting an ascertainment bias. (2) AF may be the presenting clinical phenotype in the natural

Table 2. Variants classified as likely pathogenic or pathogenic in genes linked to atrial fibrillation.

Chr (hg19)	Position (hg19)	Gene	Canonical Transcript	Exon	Nucleotide	Protein	Cardiac Transcript	Exon	Nucleotide	Protein	Variant	Classification
2	179410776	*TTN*	ENST00000589042	343/363	95187 G > C	W31729C	ENST00000460472	171/191	67992 G > C	W22664C	Missense	LP (PM2, PM5, PP3, BP1)
2	179474028	*TTN*	ENST00000589042	273/363	52009 C > T	R17337*	ENST00000460472	101/191	24814 C > T	R8272*	Nonsense	LP (PVS1, PM2)
11	2591950	*KCNQ1*	ENST00000155840	3/16	573_577del	R192Cfs*91	ENST00000155840	3/16	573_577del	R192Cfs*91	Frameshift	P (PVS1, PM1, PP5)
2	179452732	*TTN*	ENST00000589042	305/363	63399_63402del	S21134Wfs*25	ENST00000460472	133/191	36204_36207del	S12069Wfs*25	Frameshift	LP (PVS1, PM2)
2	179642033	*TTN*	ENST00000589042	27/363	4657 C > T	Q1553*	ENST00000460472	26/191	4519 C > T	Q1507*	Nonsense	LP (PVS1, PM2)
2	179606055	*TTN*	ENST00000589042	48/363	11905del	V3969Lfs*67	ENST00000460472	45/191	10816del	V3606Lfs*67	Frameshift	LP (PVS1, PM2)

Chr Chromosome, *P* Pathogenic, *LP* Likely Pathogenic.
* Translation Termination.

Table 3. Variants classified as likely pathogenic or pathogenic in genes linked to cardiomyopathy.

Chr (hg19)	Position (hg19)	Gene	Canonical Transcript	Exon	Nucleotide	Protein	Cardiac Transcript	Exon	Nucleotide	Protein	Variant	Classification
1	78399009	*NEXN*	ENST00000334785	10/13	1096 C > T	Q366*	ENST00000342754	6/10	793 C > T	Q265*	Stop Gain	LP (PVS1, PM2)
14	23886807	*MYH7*	ENST00000355349	31/40	4258 C > T	R1420W	ENST00000355349	31/40	4258 C > T	R1420W	Missense	LP (PM5, PP3)

Chr Chromosome, *P* Pathogenic, *LP* Likely Pathogenic.
* Translation Termination.

history for a proportion of channelopathies or cardiomyopathies, highlighting the need for longitudinal assessments in genes that have multiple clinical phenotypes. (3) Utilizing ACMG/AMP criteria should only be applied to AF genes with sufficient gene to disease evidence that support a monogenic cause, so we limited our efforts to genes with robust evidence, and this will need to formally curated in the near future. (4) We could not utilize all available guideline-based criteria since parental and familial segregation was not possible in this study, which may have helped to further refine the classification for some of the variants of unknown significance. This significant limitation mirrors real-world practice with adult patients where accessing parental samples may be difficult.

CONCLUSIONS

This study applied clinical diagnostic criteria for identifying monogenic causes in AF. We highlight 8 genes (*SCN5A*, *KCNA5*, *TTN*, *PITX2*, *KCNQ1*, *GJA5*, *LMNA*, *TBX5*) that contain definite, strong, and moderate literature evidence for AF. We found that 3.0% of our well-phenotyped and early-onset AF patients had a pathogenic or likely pathogenic variant in genes with robust evidence. This complements previous work completed on this cohort and suggests a combined diagnostic yield of 5.0% for identifying a monogenic explanation across genes with robust evidence in patients with early-onset AF. This not only provides new clinical insight and guidance to clinicians for genetic evaluation of AF, but it also highlights the potential clinical utility for offering different screening and treatment regimens in AF patients with an underlying monogenic defect. Further discovery work is needed to dissect the additional monogenic and polygenic determinants for high risk patients without a genetic explanation for their AF.

DATA AVAILABILITY

Data will be made available upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization: BC, AL, ZL; Data curation: BC, AL; Formal analysis: BC, AM; Investigation: BC; Resources: AL, ZL; Software: AL; Visualization: BC; Writing-original draft: BC; Writing-review & editing: BC, EL, AL, KR, MB, JW, LH, TR, JL, RH, AL, ZL.

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ETHICAL APPROVAL

We attest that the research included in this report was conducted in a manner consistent with the principles of research ethics, such as those described in the Declaration of Helsinki and/or the Belmont Report. In particular, this research was conducted with the voluntary, informed consent of any research participants, free of coercion or coercive circumstances, and received Research Ethics Board (REB) approval from the University of British Columbia that is consistent with the principles of research ethics and the legal requirements of the lead authors' jurisdiction(s). Written informed consent was obtained from all participants under a protocol approved by the University of British Columbia Research Ethics Board (H16-02531).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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