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Broad Genetic Testing in a Clinical Setting Uncovers a High Prevalence of Titin Loss-of-Function Variants in Very Early-Onset Atrial Fibrillation

William R. Goodyer, MD, PhD[1,2], **Kyla Dunn, MS**[3], **Colleen Caleshu, MS**[3], **Mary Jackson, BS**[3], **Jennifer Wylie, MS**[3], **Tia Moscarello, MS**[3], **Julia Platt, MS**[3], **Chloe Reuter, MS**[3], **Allysonne Smith, NP**[3], **Anthony Trela, NP**[2], **Scott R. Ceresnak, MD**[2], **Kara S. Motonaga, MD**[2], **Euan Ashley, MD, PhD**[1,3,4], **Phillip Yang, MD**[1,4], **Anne M. Dubin, MD, FHRS**[2], **Marco Perez, MD**[1,3,4]

[1]Cardiovascular Institute, Stanford University

[2]Division of Pediatric Cardiology, Department of Pediatrics, Lucille Packard Children's Hospital

[3]Stanford Center for Inherited Cardiovascular Disease, Stanford University School of Medicine, Stanford, CA

[4]Division of Cardiovascular Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA

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Atrial fibrillation (AF) is the most common sustained arrhythmia, affecting approximately 34 million worldwide[1]. The pathophysiology of AF remains incompletely understood but is clearly complex with multiple underlying genetic, physiologic and environmental factors. Very early-onset AF (vEAF) (defined here as onset <45 years and without significant comorbidities), while rare (only ~0.5–3% of AF cases), is highly heritable, with a greater prevalence of rare variants in genes previously associated with AF[2]. Patients with vEAF, therefore, represent an ideal population for discovering novel genes involved in the underlying genetic basis of AF. Notably, the Framingham study showed that patients with AF without comorbidities have a three-fold higher risk for heart failure[3]. Conversely, several forms of inherited cardiomyopathy have been strongly associated with AF[4] suggestive of a shared etiology.

Correspondence: Marco Perez, MD, 300 Pasteur Drive, MC 5773, Stanford, CA 94305, Tel: (650) 723-9363, Fax: (650) 725-7568, mvperez@stanford.edu.

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In this study, we investigated a rare cohort of patients with vEAF having otherwise normal cardiac structure and function using comprehensive genetics evaluations, including broad clinical genetic testing. Exclusion criteria included congenital heart disease or traditional AF risk factors including hyperthyroidism, heart failure, significant valvular disease, hypertension, diabetes mellitus, myocardial ischemia, morbid obesity, concurrent infection, alcohol, stimulant abuse, chronic obstructive pulmonary disease, and/or pulmonary hypertension. We hypothesized that the prevalence of rare genetic variation in genes associated with cardiomyopathy would be higher in this cohort.

Specifically, we assessed a cohort of consecutive patients referred to the Stanford Center for Inherited Cardiovascular Diseases for evaluation of vEAF between 2014 and 2018. This retrospective chart review was approved by the Stanford University Institutional Review Board. Patients were included if they had normal cardiac function and a structurally normal heart by initial echocardiogram as well as no other significant comorbidities. Each patient received genetic counseling and a detailed 3–4 generation family history was collected by a cardiovascular genetic counselor. We identified 25 families with vEAF. This includes 23 unrelated patients with vEAF and 2 unrelated patients with AF onset <60 years with a first-degree family member with vEAF (Probands 2 and 17). The mean age of AF diagnosis was 27.2 years (SD 13.5) and 76% of patients were male (Table). All patients at the time of their AF diagnosis had structurally normal hearts with the exception of the probands of Family 2 and 21, who initially had tachycardia-induced cardiac dysfunction but soon after exhibited restoration of normal ventricular ejection fraction following rhythm control. Notably, 40% of patients (10 of 25) had a first- or second-degree relative with vEAF, while 36% (9 of 25) had first- or second-degree relatives with either early onset (<50 years) idiopathic cardiomyopathy (20%, 5 of 25), unexplained sudden death (20%, 5 of 25) and/or strokes (12%, 3 of 25).

Patients underwent genetic testing using inherited arrhythmias and cardiomyopathy panels (73 to 149 genes) from CLIA and CAP approved commercial laboratories. The majority of patients (21 of 25) received the Arrhythmia and Cardiomyopathy Comprehensive Panel (Invitae, San Francisco. 149 genes). Variant classifications reported here are based on re-assessment by our team in 2019 using contemporary gene- and disease-specific classification approaches. Genetic testing identified at least one rare variant in a cardiomyopathy-associated gene in 85% or 21 of 25 patients, while one proband had no rare variants detected and the remaining three had rare variants in known AF-related genes. Notably, 6 of the 25 patients (24%) had actionable variants deemed “likely pathogenic” or “pathogenic”. Four of these six patients had likely pathogenic, loss-of-function (LOF) variants in the sarcomeric gene Titin (TTN) [p.Gly17311ValfsX46 (c.51930delT) in exon 241; p.Lys17359Asnfs*9 (c.52077_52078delinsT) in exon 273; p.Arg19624* (c.58870C>T) in exon 300; and p.Arg31606X (c.94816C>T) in exon 341]. Truncating A-band variants such as these are significantly overrepresented in patients with dilated cardiomyopathy and considered to be likely pathogenic for that disease. Notably, these four TTN truncation variants represented 16% of the cohort, larger than previously reported[5,6]. Additionally, another pathogenic variant was detected in another sarcomere-related gene, RBM20 (Proband 1).

To date, 11 patients have received further evaluation by MRI or CT (mean interval time after echocardiogram 817 days, SD: 1194 days), with 8 revealing reduced ventricular function, chamber enlargement, borderline LV non-compaction or late gadolinium enhancement not appreciated on presenting echocardiogram consistent with either interval disease development or possibly increased sensitivity of detection.

Overall, in a cohort of 25 patients with vEAF but otherwise normal heart structure and function at presentation, clinical genetic evaluations revealed not only a high rate of familial vEAF but also cardiomyopathy within the pedigrees. Consistently, genetic testing using expanded clinical gene panels uncovered a high burden of rare variation in cardiomyopathy-related genes, most notably in LOF, truncation variants of TTN. These results were coupled with new structural findings by cardiac MRI in some that had previously not manifested at presentation. Together these data suggest an association between vEAF and rare variants in TTN prior to the clinical onset of cardiomyopathy. While additional studies with larger clinical cohorts are clearly needed to translate these findings into clinical practice, our study would argue for a more thorough clinical evaluation and longitudinal follow up in this unique subpopulation of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms:

LOF	loss-of-function
TTN	titin
vEAF	very early onset atrial fibrillation

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Table.

Demographics and Testing Results.

Genetic variants detailed with amino acid variation only for simplicity.

Family	AF Onset (yrs)	Sex	Race/Ethnicity	Rare Genetic Variants	Variant Class	Family Hx
1	20	F	AA	**RBM20** p.Arg634Gln	**Pathogenic**	*†‡†
2	58	F	Caucasian	**TTN** p.Arg31606X	**Likely pathogenic**	*†‡†§†
3	16	M	Caucasian	**ANK2** p.Asp905Asn	VUS	
4	21	M	Caucasian	**SCN5A** p.Thr1779Met	VUS	
5	27	M	Caucasian	**FKRP** p.Ala13Thr	VUS	* †
6	40	M	Asian	**RBM20** p.Cys417Tyr	VUS	
7	43	M	Caucasian	**TTN** p.Gly17311ValfsX46	**Likely pathogenic**	
8	38	M	Caucasian	**BAG3** p.Arg45Cys	VUS	* †
9	39	M	Caucasian	**ANK2** p.Gly1439Cys	VUS, probably benign	
10	30	M	Asian	**SCN5A** p.Asp1156Gly	VUS, probably benign	
				ANK2 p.Ala373Val	VUS, probably benign	
11	16	M	Hispanic	**TTN** p.Lys17359Asnfs*9	**Likely pathogenic**	*†‡
				KCNT1 p.Pro546Leu	VUS	
				NEXN p.Tyr640Thrfs*14	VUS	
				RBM20 p.Gly583Asp	VUS, probably benign	
				SCN10A p.Val1024Met	VUS, probably benign	
12	14	M	Caucasian	**CACNA1C** p.Phe1226Leu	VUS	
				DEPDC5 p.Ala1091Val	VUS	
13	40	M	Caucasian	**ABCC9** p.Leu1524Lysfs*5	VUS	
				ALMS1 p.Arg3239Cys	VUS, probably benign	
14	30	F	Caucasian	**ALMS1** p.Ile486Val	VUS, probably benign	//
15	20	M	Caucasian	**DMD** p.Gly2609Asp	VUS	//
				FKRP p.Ser152Arg	VUS	
16	19	M	Caucasian	**KCNQ1** p.Arg231His	**Pathogenic**	* †
				LAMP2 p.Ile379Val	VUS	
17	53	M	Caucasian	**FKRP** p.Pro358Leu	VUS	*†§
				ANK2 p.Arg2506Gln	VUS	
18	44	M	Caucasian	**CAV3** p.Arg148Trp	VUS, probably benign	
19	16	M	Hispanic	**KCNA5** p.Gly182Arg	VUS, probably benign	§//
				SCN10A p.Arg814His	VUS, probably benign	
20	10	F	AA	**ACADVL** p.Glu643Asp	VUS, probably benign	*†‡
				MYBPC3 p.Gly1093Gly	VUS, probably benign	
				MYLK2 c.1425–6C>A (Intronic)	VUS	
21	25	F	Hispanic	**TTN** p.Arg19624*	**Likely pathogenic**	

Family	AF Onset (yrs)	Sex	Race/Ethnicity	Rare Genetic Variants	Variant Class	Family Hx
22	16	M	Caucasian	**DSP** p.Val495Met	VUS, probably benign	
23	17	M	Hispanic	None	None	
24	14	F	Caucasian	**ANK2** p.Ser3446Gly	VUS	*
				DTNA p.Arg536Trp	VUS	
				RYR2 c.1292+3A>G (Intronic)	VUS	
25	15	M	Caucasian	**RYR2** p.Glu4431Lys	VUS	*†‡//
				DSC2 p.Leu294Ile	VUS, probably benign	

AA: African American, AF Onset: atrial fibrillation onset of proband, VUS: variant of unknown significance

*vEAF (<45 years)

‡Cardiomyopathy (<50 years)

§Stroke (<50 years)

//Sudden Death (<50 years)

†further denotes disease process in first-degree relative.