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ORIGINAL ARTICLE

Yield of Family Screening in Arrhythmogenic Right Ventricular Cardiomyopathy Without a Validated Genetic Cause

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BACKGROUND: Current guidelines recommend regular screening for first-degree relatives of gene-elusive arrhythmogenic right ventricular cardiomyopathy (ARVC) patients using a similar regimen as for genotype-positive/phenotype-negative relatives. However, the multifactorial nature of gene-elusive ARVC may necessitate a different approach. This study aimed to determine the yield of cardiac screening in first-degree relatives of ARVC probands without a validated genetic cause.

METHODS: We included all first-degree relatives of probands who (1) met the 2010 Task Force Criteria, (2) underwent next-generation sequencing that included all genes with at least moderate evidence for ARVC causation per Clinical Genome Resource appraisal (validated ARVC genes), and (3) had no pathogenic/likely pathogenic (P/LP) variants identified in these genes. The primary and secondary end points were definite ARVC by the 2010 Task Force Criteria and ventricular arrhythmia, respectively.

RESULTS: We included 44 relatives (39.0 [22.3–45.8] years; 36% male) from 24 families. In 4 (17%) families, a P/LP variant was identified in a different cardiomyopathy/arrhythmia gene (*SCN5A*, *LMNA*, *CDH2*, *FLNC*). Overall, 10 (23%) relatives had definite ARVC at baseline evaluation. Of the 20 relatives without definite ARVC who had follow-up available, 8/20 (40%) relatives progressed to definite ARVC during 9.0 (5.8–14.4) years of follow-up. No statistical difference in the yield of baseline screening or serial evaluation between relatives from families with a P/LP variant and relatives from families without a P/LP variant was observed. Of the 27 relatives who had follow-up available, ventricular arrhythmia was observed in 2/27 (7%) relatives and occurred 6.3 and 13.8 years after definite ARVC diagnosis. Both of those relatives were from families without a P/LP variant.

CONCLUSIONS: These findings highlight the importance of managing first-degree relatives of ARVC probands without a validated genetic cause similarly to genotype-positive ARVC relatives. Furthermore, using a broad cardiomyopathy and arrhythmia gene panel in ARVC probands, rather than limiting testing to validated ARVC genes alone, is warranted.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: arrhythmogenic right ventricular dysplasia ■ genotype ■ humans ■ male ■ phenotype

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy predisposing patients to potentially life-threatening ventricular arrhythmias (VAs).[1] Current guidelines recommend performing cardiac screening in relatives for ARVC starting at age 10 to 12, with reevaluations every 1 to 3 years

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WHAT IS KNOWN?

- Gene-elusive arrhythmogenic right ventricular cardiomyopathy (ARVC) may have an auto-immune component or exercise-induced suggesting that gene-elusive ARVC may be not a genetic/heritable disease.
- Family screening in ARVC is recommended, however there is no data present on the yield of family screening in gene-elusive ARVC.

WHAT THE STUDY ADDS

- Family screening of first-degree relatives from ARVC probands without a validated ARVC gene is warranted.
- The yield of family screening of first-degree relatives from ARVC probands without a validated ARVC gene is comparable to the overall ARVC family screening population.
- ARVC probands should undergo a broad cardiomyopathy and arrhythmia gene panel, rather than limiting testing to validated ARVC genes alone.

Nonstandard Abbreviations and Acronyms

ACM	arrhythmogenic cardiomyopathy
ARVC	arrhythmogenic right ventricular cardiomyopathy
ClinGen	Clinical Genome Resource
FDR	first-degree relative
P/LP	pathogenic/likely pathogenic
TFC	Task Force Criteria
VA	ventricular arrhythmia

using an ECG, Holter monitor, and cardiac imaging (echocardiography or cardiac magnetic resonance imaging), regardless of family genotype.[2–5]

Nowadays, a causal genetic variant can be detected in approximately half of ARVC probands.[6,7] After a pathogenic or likely pathogenic (P/LP) variant is identified in a proband, cascade genetic testing can be offered to identify which relatives are at risk for developing ARVC and require longitudinal screening, while those without the P/LP variant can be safely discharged. In (near-)monogenic disease, this approach is adequate.[8] However, recent studies have shown that ARVC may have an autoimmune component[9,10] or be exercise-induced,[11] suggesting that at least a proportion of ARVC patients may not have a primarily genetic/heritable disease. As a result, relatives of those probands may not be at significant risk for ARVC, and systematic family screening will have a lower yield. Consequently, managing first-degree relatives (FDRs) of a gene-elusive ARVC proband similarly to a relative harboring the familial P/LP variant may lead to overscreening and increased anxiety in those deemed to be at potential risk.[8,12]

Although several family screening studies have been performed,[13–16] the vast majority included relatives with a P/LP variant, limiting our understanding of ARVC penetrance in relatives from gene-elusive families. To fill this gap, we aimed to quantify the yield of ARVC screening and the risk of VA in relatives from ARVC families in which no validated genetic cause was found.

METHODS

Study Population

The study population was recruited from the Johns Hopkins ARVC Registry and the Netherlands arrhythmogenic cardiomyopathy (ACM) registry.[17] From both registries, all families were identified in which the proband: (1) fulfilled definite ARVC as per the 2010 Task Force Criteria (TFC),[18] (2) had undergone next-generation sequencing that included at a minimum all genes with at least moderate evidence for ARVC-causation per the most recent Clinical Genome Resource (ClinGen) evidence-based appraisal,[19] and (3) had no P/LP variant identified in these genes, as described below. From the families identified, we then included all FDRs of ARVC patients who had undergone a baseline evaluation allowing for ascertainment of fulfillment of definite ARVC per the TFC. This study followed the Code of Conduct for the Use of Data in Health Research and was approved by local ethics and institutional review boards. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Genetic Testing

For the purpose of this study, to be considered a gene-elusive family, the proband had to have undergone next-generation sequencing with no P/LP variant detected in any gene with at least moderate evidence of ARVC causation (ie, Plakophilin-2 [*PKP2*], Desmoplakin [*DSP*], Desmoglein-2 [*DSG2*], Desmocollin-2 [*DSC2*], Junctional plakoglobin [*JUP*], Transmembrane protein-43 [*TMEM43*], Phospholamban [*PLN*], and Desmin [*DES*]).[19] In the article, we will refer to these genes as validated ARVC genes, whereas other genes, including those with limited evidence for ARVC causation as per ClinGen appraisal, will be referred to as nonvalidated ARVC genes. Genetic testing panels were selected at the discretion of the genetic counselor or treating cardiologist. All genetic test results were adjudicated by an experienced cardiac genetic counselor according to the American College of Medical Genetics criteria (B.M.).[20,21] In addition, P/LP variants in nonvalidated ARVC genes were reported along with the initial presentation of the proband and the results of cascade genetic testing to provide data for a potential reclassification.

Clinical Evaluation

Participants were evaluated as described previously.[14,22] The medical history of each relative was obtained by review of electronic medical records. Detailed clinical information regarding demographics, presentation, symptom onset, and (non-)

invasive tests was obtained for every participant. Pedigree analysis was performed by genetic counselors with expertise in ARVC. Relatives were divided based on their relationship to the proband as parents, siblings, and children.

All relatives underwent guideline-recommended baseline screening evaluation in which definite ARVC diagnosis could be ascertained, defined as a 12-lead ECG, a Holter monitor of at least 24 hours, and an imaging modality (echocardiogram or cardiac magnetic resonance imaging).[2–5] Testing results from other modalities (eg, angiogram) were also collected. For follow-up evaluations, we included all available clinical testing performed at the discretion of the treating cardiologist, including a 12-lead ECG, Holter monitoring of at least 24 hours, and imaging (echocardiogram or cardiac magnetic resonance imaging).

ARVC Diagnosis

Diagnosis of ARVC was based on the 2010 TFC.[18] Within this framework, definite ARVC diagnosis is defined as fulfilling 2 major, 1 major plus 2 minor, or 4 minor criteria. By study design, all relatives fulfilled 1 major criterion in the family history category given their relationship to the proband. As such, relatives were stratified by their baseline clinical phenotype: clinically unaffected (ie, only the major family history criterion without any TFC fulfillment on diagnostic tests), borderline ARVC (ie, fulfillment of 1 minor criterion plus the major family history criterion), or definite ARVC (ie, fulfillment of at least 2 minor or 1 major plus the major family history criterion).

Study Outcomes

The primary outcomes of this study were (1) definite ARVC diagnosis as per 2010 TFC at baseline and follow-up; and (2) fulfillment of previously unfulfilled TFC during follow-up. Secondary outcomes were the occurrence of sustained VA. Sustained VA was defined as a composite of sudden cardiac death, sudden cardiac arrest, spontaneous sustained ventricular tachycardia (ventricular tachycardia lasting ≥30 seconds at ≥100 bpm and with hemodynamic compromise requiring cardioversion), ventricular fibrillation, or appropriate Implantable Cardioverter Defibrillator intervention, as reported previously.[14,22]

Statistical Analysis

Nominal variables were expressed as number (%), and continuous variables as mean±SD or median (interquartile range), as appropriate. Comparisons for binary variables were performed by ANOVA, χ^2, or Fisher exact test, as appropriate. For continuous variables, an independent 2-sample *t* test or Mann-Whitney *U* test was used, as appropriate. Distribution plots and Kaplan-Meier curves were used to visualize the primary end point at baseline and during follow-up, respectively. Next, we stratified our population based on the presence/absence of a familial P/LP variant in a nonvalidated ARVC gene and plotted Kaplan-Meier curves to visualize the rate of progression. A $P<0.05$ was considered statistically significant. Data were analyzed using R, version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study Population

In total, 24 families met the inclusion criteria for having ARVC without a validated genetic cause, including 63 FDRs who enrolled in one of the ACM registries. Of those 63 FDRs, 44 (69%) individuals had undergone a complete baseline evaluation (Figure 1 and Figure S1 for stratification based on P/LP variants in nonvalidated ARVC genes). Baseline characteristics are shown in Table 1. Median age at first evaluation was 39.0 (22.3–45.8) years and 16 (36%) were male. Overall, most relatives were asymptomatic at baseline (n=37/44, 84%), while the remaining 16% (n=7/44) reported palpitations. No presyncope or syncope was reported. Of note, we included 2 relatives who were second-degree relatives of the proband; both of these relatives had an FDR who also fulfilled definite ARVC diagnosis.

Presence of P/LP Variants in Nonvalidated ARVC Genes

Genetic testing outside of the ClinGen definition of moderate- or definite-evidence genes for ARVC was performed in all families to varying extents. As shown in Table S1, 2 probands underwent whole-exome sequencing, and the remaining 22 probands had a median of 66 (62–78) genes tested. In 4 of 24 (17%) families, a P/LP variant was detected in genes classified as having limited or no evidence for ARVC causation (ie, nonvalidated ARVC genes). As shown in Table 2, all 4 probands

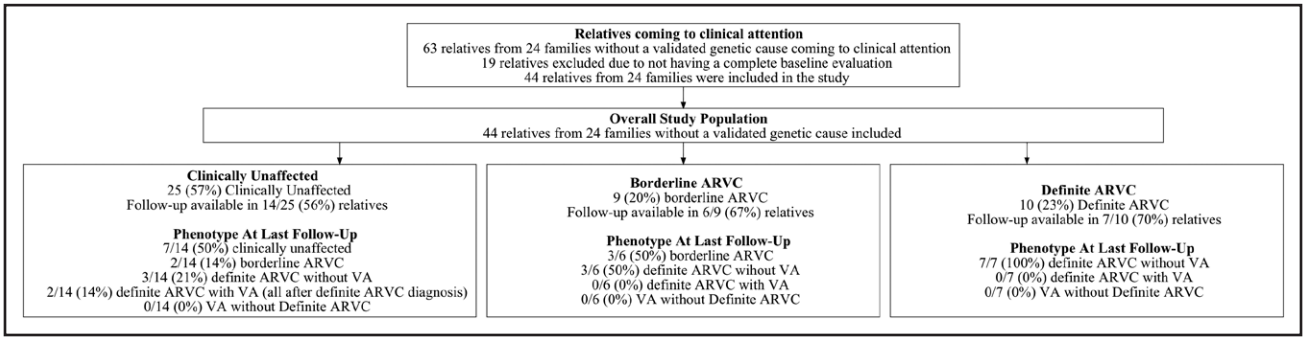

Figure 1. Study flowchart.
ARVC indicates arrhythmogenic right ventricular cardiomyopathy; and VA, ventricular arrhythmia.

Table 1. Baseline Characteristics

	Overall (N=44)	Clinically unaffected (N=25)	Borderline ARVC (N=9)	Definite ARVC (N=10)	*P* value
Age at presentation, y	39.0 (22.3–45.8)	38.7 (17.8–45.7)	26.9 (24.0–42.0)	42.5 (34.9–50.5)	0.298
Male	16 (36.4)	8 (32.0)	5 (55.6)	3 (30.0)	0.402
Relationship to the proband					0.578
Sibling	16 (36.4)	7 (28.0)	3 (33.3)	6 (60.0)	
Child	18 (40.9)	11 (44.0)	5 (55.6)	2 (20.0)	
Parent	8 (18.2)	5 (20.0)	1 (11.1)	2 (20.0)	
2nd degree*	2 (4.5)	2 (8.0)	0 (0.0)	0 (0.0)	
Any symptoms	7 (15.9)	1 (4.0)	3 (33.3)	3 (30.0)	0.022
P/LP variant in family					0.402
No	33 (75.0)	19 (76.0)	7 (77.8)	7 (70.0)	
Yes, also in relative	6 (13.6)	2 (8.0)	1 (11.1)	3 (30.0)	
Yes, not in relative	5 (11.4)	4 (16.0)	1 (11.1)	0 (0.0)	
Electrical TFC fulfillment	19 (43.2)	0 (0.0)	9 (100.0)	10 (100.0)	<0.001
Repolarization TFC fulfillment	10 (22.7)	0 (0.0)	3 (33.3)	7 (70.0)	<0.001
T-wave inversions V1–V2	5 (11.4)	0 (0.0)	3 (33.3)	2 (20.0)	
T-wave inversions V1–V3	4 (9.1)	0 (0.0)	0 (0.0)	4 (40.0)	
T-wave inversions V4–V6	1 (2.3)	0 (0.0)	0 (0.0)	1 (10.0)	
Depolarization TFC fulfillment	10 (22.7)	0 (0.0)	5 (55.6)	5 (50.0)	<0.001
Prolonged TAD	4 (9.1)	0 (0.0)	2 (22.2)	2 (20.0)	0.029
Late potentials on SAECG (N=27)	6 (35.3)	0 (0.0)	3 (60.0)	3 (60.0)	0.039
Holter TFC fulfillment	8 (18.2)	0 (0.0)	1 (11.1)	7 (70.0)	<0.001
PVC count/24 h	2 (0–58)	1 (0–13)	0 (0–3)	1077 (193–2785)	0.005
Imaging TFC fulfillment	1 (2.3)	0 (0.0)	0 (0.0)	1 (10.0)	0.176
CMR TFC fulfillment (N=19)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	…
Presence of RV WMAs (N=19)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	…
RVEDV/BSA, mL/m² (N=19)	89.5 (73.6–100.5)	81.1 (67.0–95.0)	92.2 (77.8–102.5)	100.2 (88.4–112.0)	0.507
RVEF (%; N=19)	51.0 (47.9–55.0)	53.5 (49.8–56.0)	48.0 (47.6–50.3)	55.5 (53.3–57.8)	0.292
LVEF (%; N=19)	61.0 (57.5–64.5)	61.0 (61.0–65.0)	60.0 (57.5–64.8)	52.00 (49.50–57.50)	0.286
Echocardiographic TFC fulfillment (N=43)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	…
Presence of RV WMAs (N=43)	2 (4.7)	0 (0.0)	1 (12.5)	1 (10.0)	0.226
RVOT PLAX/BSA, mL/m² (N=7)	14.9 (14.4–17.5)	16.0 (14.6–17.3)	12.2 and 17.4	14.9	1.000
RVOT PSAX/BSA, mL/m² (N=2)	16.4 and 17.1	16.4 and 17.1	…	…	…
Angiographic TFC fulfillment (N=1)	1 (100.0)	…	…	1 (100.0)	…

Variables are expressed as frequency (%) or median (IQR). Total number of patients for a given variable are mentioned if missing data. For categorical and continuous variables, we performed ANOVA and Mann-Whitney *U* test, respectively. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; BSA, body surface area; LVEF, left ventricular ejection fraction; P/LP, pathogenic/likely pathogenic; PLAX, parasternal long axis; PSAX, parasternal short axis; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; RVOT, right ventricular outflow tract; SAECG, signal-averaged ECG; TAD, terminal activation duration; and WMA, wall motion abnormalities.

*Both second-degree relatives had a first-degree relative who had definite ARVC diagnosis and therefore had a screening indication for ARVC.

fulfilled the 2010 TFC irrespective of a family history. Cascade genetic testing revealed that 6 of 11 (55%) relatives from those 4 families harbored the familial P/LP variant, and 5 of 11 (45%) proved not to harbor the familial variant (Figure 1).

Baseline Cardiac Evaluation

At first cardiac evaluation, 25 (57%) relatives were clinically unaffected, 9 (20%) had borderline ARVC, and 10 (23%) had definite ARVC diagnosis. Relatives who were symptomatic were significantly more likely to have borderline or definite ARVC (clinically unaffected: 1 [4%] versus borderline ARVC: 3 [33%] versus definite ARVC: 3 [30%]; *P*=0.022) as compared with those who were asymptomatic. Age, sex, and relationship to the proband did not statistically differ between baseline clinical phenotypes (*P*>0.05; Table 1). Figure 2 visualizes the yield of screening for different groups at baseline. As can be appreciated, the baseline yield of screening among

Table 2. Characteristics at Initial Presentation of Probands With a Pathogenic/Likely Pathogenic Variant in a Nonvalidated ARVC Gene

Proband ID, sex	Genetic variant, ACMG classification	ACMG criteria applied	Age at presentation	ECG at presentation	Imaging at presentation	Arrhythmia at presentation
1, Male	*SCN5A* c.2184_2186del; p.Leu729del LP	PS4, PM2, PM4, PP1	23 y; SCA while playing soccer	SR, PQ 188 ms, IVCD (TAD prolonged but not typical); 6 mo after SCA: TWI V1–V2	No LV/RV abnormalities	EP study: VF, polymorphic VT, and VT LBBB superior axis
2, Female	*LMNA* c.466C>T; p.Arg156Cys LP	PM1, PP2, PM2, PP3	38 y; SCD while sleeping. ARVC diagnosed by autopsy	Not available	Not available	Not available
3, Female	*CDH2* c.2035C>G; p.Pro679Ala LP	PM2, PP1, PP3, PP4, PP5	20 y; multiple syncope's during exercise	SR; PQ 144 ms; no prolonged TAD; TWI V1–V5	Mild RV dilatation with dyskinesia; RVEF 34%. No global LV abnormalities	Holter monitor: 4500 PVCs/24 h
4, Female	*FLNC* c.3307_3313del; p.C1103Pfs*84 LP	PVS1, PM2, PP5	13 y; VT LBBB unknown axis while playing basketball	Not available (no ECGs before EP study present. After EP study she had a persistent RBBB)	RV: aneurysmatic, severely enlarged, function severely decreased; LV: enlarged, LVEF 25%	EP study: late potentials and inducible clinical VT

ACMG indicates American College of Medical Genetics; ARVC, arrhythmogenic right ventricular cardiomyopathy; EP, electrophysiology; FLNC, Filamin C; IVCD, intraventricular conduction delay; LBBB, left bundle branch block; LV, left ventricular; LVEF, left ventricular ejection fraction; RV, right ventricular; SCA, sudden cardiac arrest; SR, sinus rhythm; TAD, terminal activation duration; TWI, T-wave inversions; VF, ventricular fibrillation; and VT, ventricular tachycardia.

relatives from families without a P/LP variant was 21% (n=7/33). Relatives with a P/LP variant in a nonvalidated ARVC gene had a higher yield of screening as compared with relatives from families without a P/LP variant, albeit not reaching statistical significance (definite ARVC among relatives with a P/LP variant: 3/6 [50%] versus definite ARVC from families with no P/LP variant: 7/33 [21%]; *P*=0.163).

Serial Cardiac Evaluation

Of the 34 relatives without definite ARVC diagnosis at baseline, 20 (59%) had follow-up data available. No significant differences between those with and without follow-up were present (Table S2; *P*>0.05). Median follow-up in the 20 relatives was 9.0 (5.8–14.4) years. Individual disease trajectories and group summaries are visualized in Figures 3 and 4, respectively.

Progression to New TFC

Overall, 10 of 20 (50%) relatives without definite ARVC diagnosis at baseline progressed to a new TFC (Figure 4A). Median time to new TFC was 5.4 (3.0–8.3) years and was most often observed on ECG (5/10; 50%), followed by imaging and signal-averaged ECG (both 2/10; 20%), and Holter monitoring (1/10; 10%; Figure 3). Relatives from families without a P/LP variant had similar rates of progression to new TFC as compared with the overall cohort (Figure S2A).

Progression to Definite ARVC

In total, 8 of 20 (40%) relatives progressed to definite ARVC diagnosis (Figure 4B). Median time to definite ARVC diagnosis was 7.6 (4.3–9.9) years. Relatives from families without a P/LP variant had similar rates of progression to definite ARVC as compared with the overall cohort (Figure S2B). At 5 years' follow-up, only 1 relative progressed to definite ARVC and was from a family that harbored a P/LP variant. All other relatives who progressed to definite ARVC at 5 years' follow-up were from families without a P/LP variant. This 1 relative from a family with a P/LP variant who progressed to definite ARVC during follow-up did, however, not harbor the familial P/LP *CDH2* variant. At follow-up evaluation, she was found to have T-wave inversions in leads V1 to V3, while showing no other phenotypic expression on other diagnostic tests (no premature ventricular complexes nor structural abnormalities) during 4.0 years of follow-up. She had a history of hypertension and a body mass index of ≥30 kg/m^2, possibly suggesting a different cause of her T-wave inversions than ARVC. In contrast, the relative who did harbor the familial *CDH2* variant experienced clear phenotypic expression of ARVC on ECG (T-wave inversions in leads V1–V3 and >500 premature ventricular complexes/24 hours on Holter monitoring).

Risk of VA

In addition to the 20 relatives without definite ARVC diagnosis at baseline, 7 relatives who had definite ARVC diagnosis at baseline had follow-up available, leading to a total of 27 relatives with follow-up evaluation. During a median follow-up of 10.3 (6.3–13.9) years, 2/27 (7%) relatives experienced a VA: 1 had monomorphic ventricular tachycardia with a cycle length of 245 ms terminated by an Implantable Cardioverter Defibrillator shock; and 1 experienced sudden cardiac arrest terminated by an external defibrillator. Both relatives were from families without a P/LP variant. In addition, neither relative

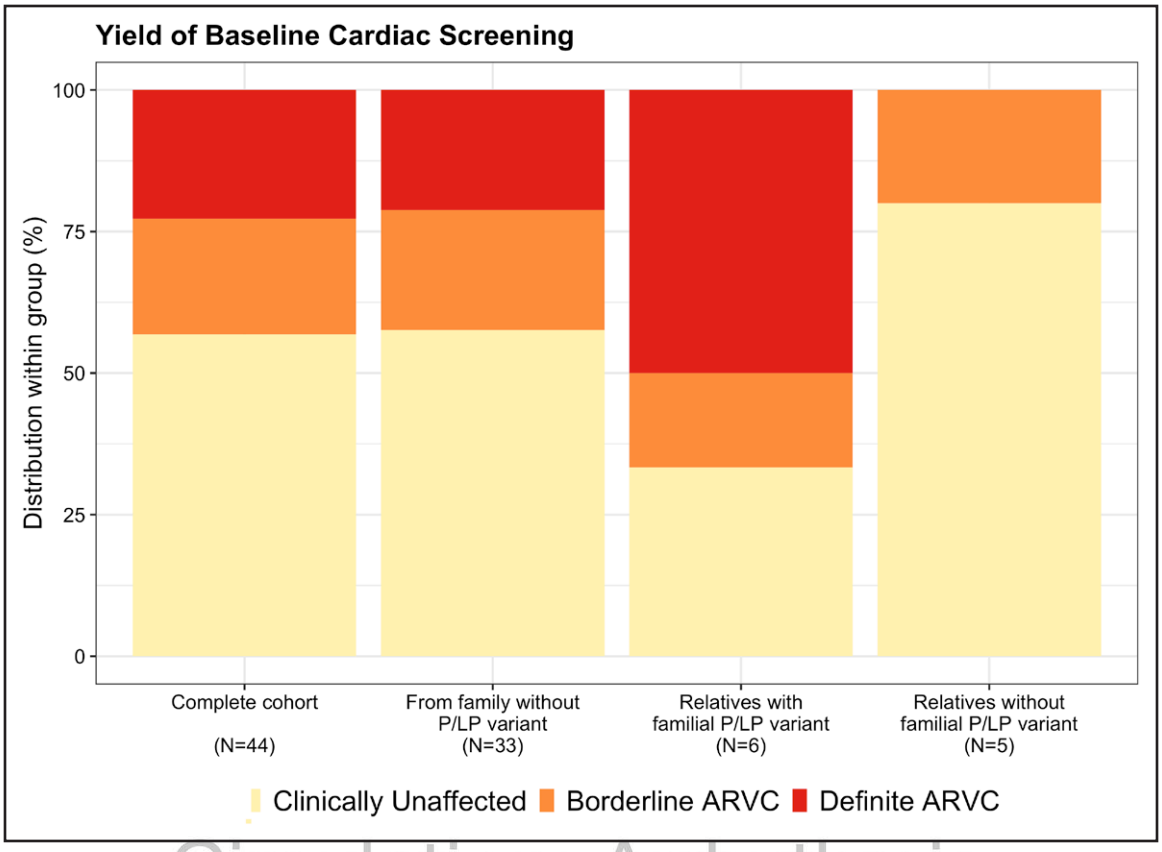

Figure 2. Yield of baseline cardiac screening.
Prevalence of definite arrhythmogenic right ventricular cardiomyopathy (ARVC) among first-degree relatives at time of baseline cardiac evaluation. To show the proportion of relatives in each group, every group is scaled to 100%. The number of patients included in every bar is denoted in the *x* axis. Yellow, orange, and red colors represent clinically unaffected, borderline ARVC, and definite ARVC, respectively. P/LP indicates pathogenic/likely pathogenic; and SAECG, signal-averaged ECG.

fulfilled definite ARVC diagnosis at baseline cardiac evaluation and experienced a VA at 16.3 and 23.2 years after baseline evaluation and 6.3 and 13.8 years after definite ARVC diagnosis, respectively (Figure 3). Consequently, all VAs occurred after definite ARVC diagnosis.

DISCUSSION

In the era of precision medicine and the rise of gene-centered diagnosis and management strategies,[23] assessing the efficacy of continued screening in cardiomyopathy families without an identified genetic cause is necessary. Autoimmune and exercise-induced causes of ARVC have been suggested,[9–11] and therefore demonstrating that relatives from gene-elusive families are at risk of developing ARVC is of utmost clinical importance. Our study provides evidence for continued screening of FDRs, as ARVC without a validated genetic cause is not necessarily nonheritable. Furthermore, family screening in gene-elusive ARVC families identified individuals who both progressed to definite ARVC diagnosis and developed VA events during follow-up, affirming the relevance of serial cardiac screening in FDRs. These results also support the use of expanded genetic testing in probands that include both validated ARVC-associated genes, newly identified genes associated with ARVC, as well as genes associated with ARVC phenocopies.[24–26]

Yield of Baseline Cardiac Screening

The baseline yield of family screening in an overall ARVC population has been reported to be 33%.[13–15] As mentioned earlier, these studies included primarily relatives who carry P/LP variants in a validated ARVC gene. We previously reported a meta-analysis comparing the penetrance of ARVC in FDRs of gene-elusive ARVC patients as compared with genotype-positive relatives, and found that genotype-positive FDR were 6.9 times more likely to have definite ARVC diagnosis as compared with gene-elusive relatives.[12] However, only 1 out of 3 studies in that analysis included relatives who had a complete evaluation, had genetic testing performed in the next-generation sequencing era, and excluded second-degree relatives. Indeed, the comparison in that 1 study did not show a significant difference in yield of baseline screening between genotype-positive and genotype-negative relatives.[27]

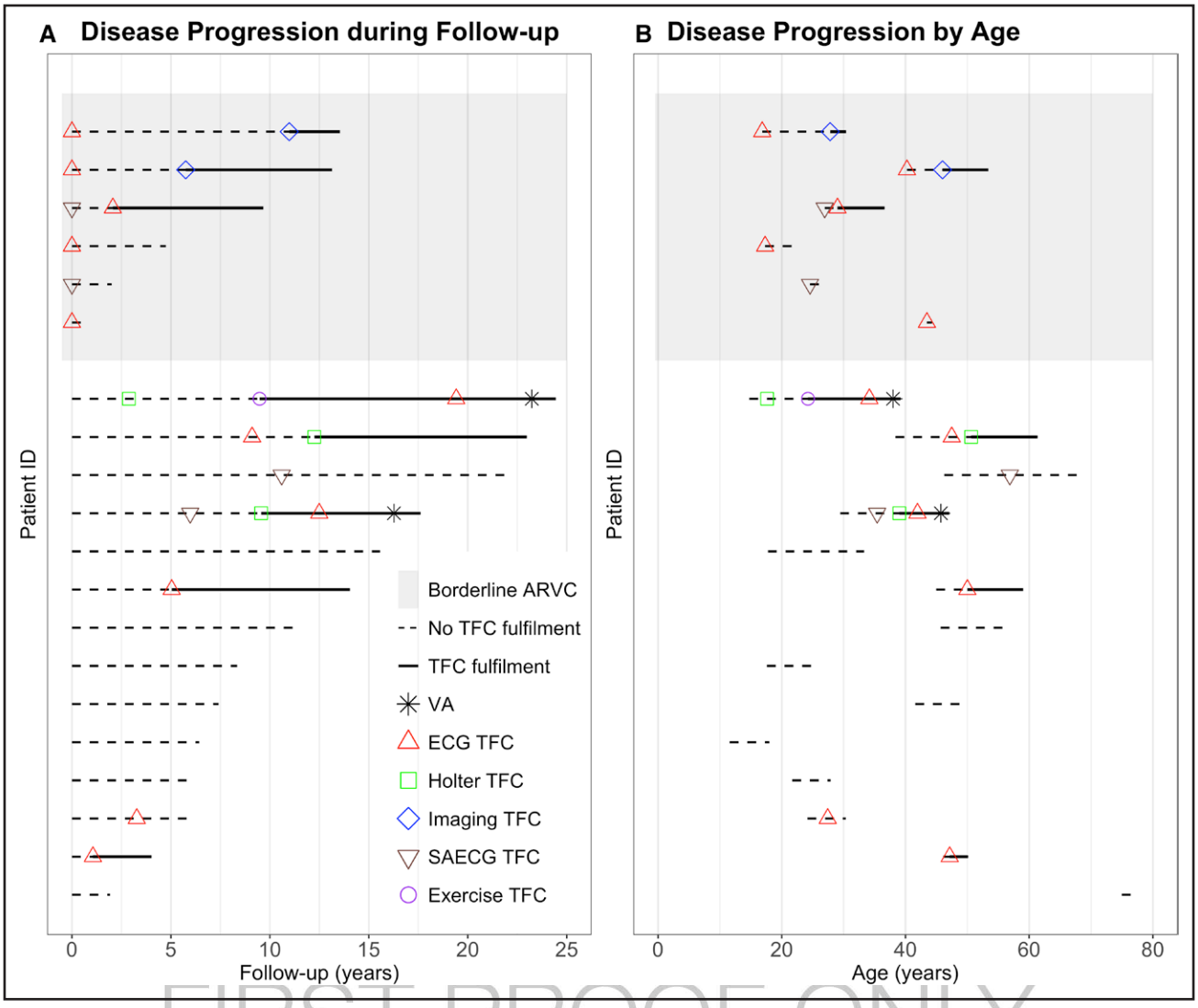

Figure 3. Disease trajectory in relatives without definite arrhythmogenic right ventricular cardiomyopathy (ARVC) at baseline cardiac evaluation.

Clinical course of all relatives not fulfilling 2010 Task Force Criteria (TFC) at baseline cardiac evaluation. **A**, Disease progression during follow-up. **B**, Disease progression by age. Each relative is displayed as a straight line. Straight lines inside the gray rectangle indicate relatives with borderline ARVC at baseline, relatives outside the gray rectangle indicate clinically unaffected at baseline. A dashed line indicates follow-up without definite ARVC diagnosis, while a solid line indicates follow-up with definite ARVC diagnosis. The initiation of each line represents first clinical evaluation. The junction between the dashed and solid lines indicates date of diagnosis. A red triangle (ECG), green square (Holter monitor), blue diamond (imaging test), brown upside-down triangle (single-averaged ECG) and purple star (exercise test) indicate new TFC during follow-up. An asterisk visualizes the occurrence of ventricular arrhythmia.

Similarly, the baseline yield in FDRs in a gene-elusive ARVC family found in our study is ≈1 in 4 at a median age of 39.0 (22.3–45.8) years, which is not far off from the overall ARVC population (1 in 3) at a similar age.[13–15] Thus, this study reinforces the importance of initiating family screening in FDRs even from gene-elusive families.

Yield of Serial Cardiac Screening

Regarding serial evaluation, 40% (n=8/20) of our study population progressed to definite ARVC diagnosis during a median follow-up of 9.0 (5.8–14.4) years. When comparing this yield to historic (genotype-positive) family screening studies,[13–15] the rate of progression to definite ARVC in FDRs from gene-elusive ARVC patients is comparable. This suggests that the guideline-recommended screening intervals of 1 to 3 years[2–5] and proposed improvements of these guideline recommendations[14] are adequate in this population. Nonetheless, it should be highlighted that ARVC without a validated genetic cause is a heterogeneous group caused by inflammation, exercise, and genetic factors.[28] For example, family members from a proband with exercise-induced ARVC might be managed differently (eg, no screening necessary if the family member

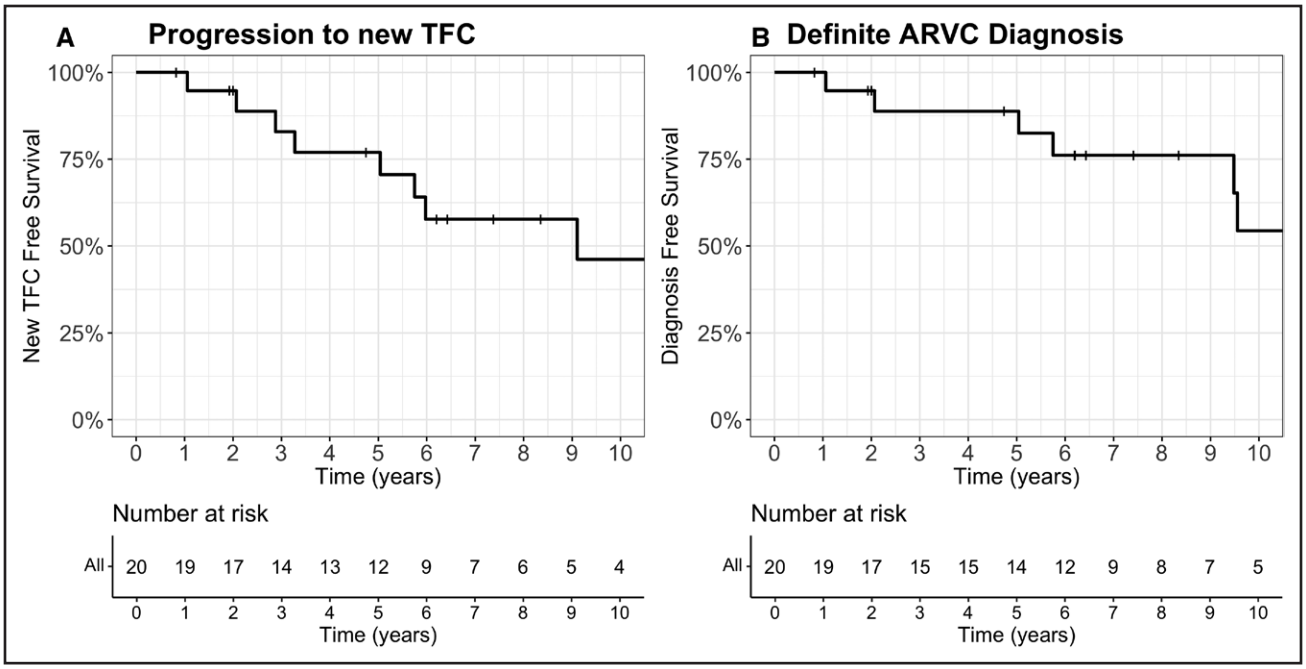

Figure 4. Yield of serial cardiac evaluation.
Survival curve of (**A**) a new Task Force Criteria (TFC) criterion and (**B**) definite arrhythmogenic right ventricular cardiomyopathy (ARVC) diagnosis during follow-up.

in question does not exercise as the trigger for ARVC development is not present) as compared with families with a more genetic cause. As such, further research is warranted to stratify the different etiologies of gene-elusive ARVC and their potential influence on family screening.[28]

In addition, our study shows the importance of a family history of ARVC when considering penetrance in ARVC. Indeed, a recent study[29] showed a 3% penetrance of ARVC in a genome-first population with definite and moderate P/LP ARVC genes (ie, genetic causation for ARVC found by accident). Consequently, comparing the 3% to the 41% (n=18/44) penetrance of ARVC at last follow-up reported in our gene-elusive cohort indicates that the presence of a family history of ARVC is a stronger predictor for ARVC development than the presence of a P/LP variant in a desmosomal gene itself.[29]

Risk of VA

The central objective of family screening is to enable early diagnosis, risk stratification, and ultimately prevention of sudden cardiac death. Similar to previous studies,[13–15] we found a low rate of VA (7%; n=2/27) after a long follow-up (16.3 and 23.2 years after baseline evaluation), and all occurred well after definite ARVC diagnosis (6.3 and 13.8 years, respectively). Indeed, our findings align with the growing body of evidence that phenotypic expression is a prerequisite for arrhythmic events in relatives with *PKP2*-associated ARVC.[13–16]

Scope of Genetic Testing

Genetic testing in ARVC patients has been guideline-recommended since the discovery of the first desmosomal genes.[18] Indeed, a recent reappraisal of genes associated with ARVC has identified 8 genes definitely or moderately associated with ARVC, established other genes that are associated with other phenotypically overlapping cardiomyopathies/arrhythmia syndromes (eg, *LMNA*), and found several genes in classic ARVC families but with limited evidence.[19] It is clear that the genetic underpinnings of ARVC and other arrhythmogenic cardiomyopathies are rapidly evolving, and therefore broad panels and consideration of expanded genetic testing for probands who were gene-elusive on an initial small panel are warranted. We found that in 17% (4/24) of ARVC families without P/LP variants in the ClinGen-defined moderate and definite ARVC genes, a P/LP variant in another gene was present.

Specifically, this study adds to the body of evidence that further reappraisal of evidence by the ClinGen ARVC GCEP may be needed. However, caution is needed as the proband with a P/LP *LMNA* variant presented with a sudden cardiac death and was diagnosed with ARVC at autopsy. Although a carrier of a P/LP *LMNA* variant can fulfill definite ARVC diagnosis, the clinical phenotype and risk stratification usually differ (eg, atrioventricular and intraventricular conduction disorders, high risk of congestive heart failure) from ARVC.[26] Likewise, genes such as *SCN5A*, *LMNA*, *CDH2*, and *FLNC* fall within the spectrum of ACMs, but there are insufficient data to indicate

that the phenotypes of these ACMs are the same as those with classical ARVC. As the scope of phenotype in all ACMs may have considerable overlap, this reinforces that broad genetic testing is recommended to ensure appropriate gene-based management in these families.[7]

Study Limitations

This study is limited by referral bias, as relatives who may have more penetrant familial disease may be more inclined to undergo family screening, which previously has been observed in long-QT syndrome.[30] Consequently, our results may be an overestimation of the true penetrance of ARVC in FDR from gene-elusive ARVC patients. In addition, relatives with more symptoms may be more likely to seek tertiary evaluation at ARVC expert centers and enroll in ARVC-related registries. Furthermore, follow-up visits, as well as the diagnostic tests, were performed at the discretion of the treating cardiologist. As such, we cannot rule out ascertainment bias for nondiagnostic abnormalities as well as stochastic events. Next, all included relatives came from a family in which we confirmed definite ARVC diagnosis in the proband. Indeed, family screening should only be performed when the diagnosis of ARVC in the proband is confirmed to prevent unnecessary screening in families with a misdiagnosis of ARVC.[31] Nevertheless, our study establishes that, regardless of a negative genetic test result in the proband, FDR should undergo cardiac screening, as a proportion of FDRs indeed develop definite ARVC diagnosis. Notably, the scope of genetic testing was ordered at the discretion of the medical team. Hence, we cannot rule out that some families may harbor an undetected P/LP variant. Although our results yielded a nonsignificant difference between relatives from a family with and without a P/LP variant, caution when interpreting these results is warranted as these results are very likely limited by a sample size resulting in a type II statistical error. Finally, age, sex, and exercise intensity have been convincingly shown to influence penetrance in ARVC, which we were not able to evaluate as risk factors in this study due to our limited sample size and event rate.[11] Future studies are necessary to determine predictors and influence of exercise on disease penetrance and outcomes in the gene-elusive ARVC population.

Conclusions

This study evaluated the yield of cardiac screening in FDR from ARVC patients without a validated genetic cause. Although the penetrance reported here is lower as compared with historic ARVC cohorts, this study reinforces the importance of cardiac screening in FDR from gene-elusive ARVC probands as ARVC diagnosis and VA events do occur in these families. In addition, our study supports that effective genetic testing in ARVC index patients should be broad including all cardiomyopathy/arrhythmia genes.

ARTICLE INFORMATION

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Supplemental Material

Tables S1–S2
Figures S1–S2

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