

## Individualized Family Screening for Arrhythmogenic Right Ventricular Cardiomyopathy

Steven A. Muller, MD, Alessio Gasperetti, MD, Laurens P. Bosman, MD, PhD, Amand F. Schmidt, PhD, Annette F. Baas, MD, PhD, Ahmad S. Amin, MD, PhD, Arjan C. Houweling, MD, PhD, Arthur A.M. Wilde, MD, PhD, Paolo Compagnucci, MD, Mattia Targetti, MD, Michela Casella, MD, PhD, Leonardo Calò, MD, Claudio Tondo, MD, PhD, Pim van der Harst, MD, PhD, Folkert W. Asselbergs, MD, PhD, J. Peter van Tintelen, MD, PhD, Marish I.F.J. Oerlemans, MD, PhD, Anneline S.J.M. Te Riele, MD, PhD

PII: S0735-1097(23)05543-2

DOI: <https://doi.org/10.1016/j.jacc.2023.05.005>

Reference: JAC 30227

To appear in: *Journal of the American College of Cardiology*

Received Date: 19 December 2022

Revised Date: 30 March 2023

Accepted Date: 5 May 2023

Please cite this article as: Muller SA, Gasperetti A, Bosman LP, Schmidt AF, Baas AF, Amin AS, Houweling AC, Wilde AAM, Compagnucci P, Targetti M, Casella M, Calò L, Tondo C, van der Harst P, Asselbergs FW, Peter van Tintelen J, Oerlemans MIFJ, Te Riele ASJM, Individualized Family Screening for Arrhythmogenic Right Ventricular Cardiomyopathy, *Journal of the American College of Cardiology* (2023), doi: <https://doi.org/10.1016/j.jacc.2023.05.005>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

# Individualized Family Screening for Arrhythmogenic Right Ventricular Cardiomyopathy

## Brief title: Individualized Family Screening for ARVC

Steven A. Muller, MD,<sup>a,b,c</sup> Alessio Gasperetti, MD,<sup>a,d,e</sup> Laurens P. Bosman, MD, PhD,<sup>a,c</sup>  
Amand F. Schmidt, PhD,<sup>f,g</sup> Annette F. Baas, MD, PhD,<sup>c,h</sup> Ahmad S. Amin, MD, PhD,<sup>c,g</sup>  
Arjan C. Houweling, MD, PhD,<sup>c,i</sup> Arthur A.M. Wilde, MD, PhD,<sup>c,g</sup> Paolo Compagnucci,  
MD,<sup>j</sup> Mattia Targetti, MD,<sup>k</sup> Michela Casella, MD, PhD,<sup>j</sup> Leonardo Calò, MD,<sup>l</sup> Claudio  
Tondo, MD, PhD,<sup>e,m</sup> Pim van der Harst, MD, PhD,<sup>a,c</sup> Folkert W. Asselbergs, MD, PhD,<sup>c,f,g,n</sup> J.  
Peter van Tintelen, MD, PhD,<sup>b,c,h</sup> Marish I.F.J. Oerlemans, MD, PhD,<sup>a</sup> Anneline S.J.M. Te  
Riele, MD, PhD<sup>a,b,c\*</sup>

## Author affiliations

<sup>a</sup>Department of Cardiology, University Medical Center Utrecht, Heidelberglaan 100, 3584  
CX Utrecht, Netherlands; <sup>b</sup>Netherlands Heart Institute, Utrecht, Moreelsepark 1, 3511 EP  
Utrecht, Netherlands; <sup>c</sup>Member of the European Reference Network for rare, low prevalence  
and complex diseases of the heart: ERN GUARD-Heart' (ERN GUARDHEART;  
<http://guardheart.ern-net.eu>); <sup>d</sup>Division of Medicine, Department of Cardiology, Johns  
Hopkins University, Baltimore, MD, US; <sup>e</sup>Department of Clinical Electrophysiology &  
Cardiac Pacing, Centro Cardiologico Monzino, IRCCS, Milano, Italy; <sup>f</sup>Institute of  
Cardiovascular Science, Faculty of Population Health Sciences, University College London,  
London, UK; <sup>g</sup>Amsterdam University Medical Centers, Department of Cardiology,  
University of Amsterdam, Amsterdam, The Netherlands; <sup>h</sup>Department of Genetics,  
University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands;

<sup>i</sup>Amsterdam University Medical Centers, Department of Human Genetics, University of Amsterdam, Amsterdam, The Netherlands; <sup>j</sup>Cardiology and Arrhythmology Clinic, University Hospital “Ospedali Riuniti”, Ancona, Italy; <sup>k</sup>Cardiomyopathy Unit, Careggi University Hospital, Florence, Italy; <sup>l</sup>Department of Cardiology, Policlinico Casilino, Rome, Italy; <sup>m</sup>Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; <sup>n</sup>Health Data Research UK and Institute of Health Informatics, University College London, London, United Kingdom.

**Funding:** We acknowledge the support from the Netherlands Cardiovascular Research Initiative, an initiative with support of the Netherlands Heart Foundation, grant nos.: CVON2015-12 eDETECT and 2020B005 Double Dose. Predict 2. The Netherlands ACM Registry is supported by the Netherlands Heart Institute (project 06901). ASJMtR is supported by the ZonMW Off Road Grant 2021. FWA is supported by UCL Hospitals NIHR Biomedical Research Centre. AFS is supported by BHF grant PG/18/5033837, PG/22/10989, and the UCL BHF Research Accelerator AA/18/6/34223.

**Disclosures:** AG has served as part of the advisory board of LEXEO Therapeutics for unrelated work; AFS has received funding from NewAmsterdam for unrelated work. The remaining authors have nothing to disclose.

**Address for correspondence:** Anneline SJM te Riele; Mailing address: Department of Cardiology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, Netherlands. Email: [ariele@umcutrecht.nl](mailto:ariele@umcutrecht.nl).

**Tweet:** The interval of familyscreening in at-risk ARVC relatives can be optimized using simple baseline characteristics, and with shareddecision making an optimal screening interval can be determined.

**Acknowledgments:** The authors thank Lian Rekker and all PhD students for data collection.

We thank the ARVC relatives who have made this work possible.

Journal Pre-proof

**Abstract**

**Background:** Clinical guidelines recommend regular screening for Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) to monitor at-risk relatives, resulting in a significant burden on clinical resources. Prioritizing relatives on their probability of developing definite ARVC may provide more efficient patient care.

**Objective:** Determine predictors and probability of ARVC development over time among at-risk relatives.

**Methods:** We included 136 relatives (46% male, 25.5 (interquartile range (IQR):15.8-44.4) years) from the Netherlands ACM Registry without definite ARVC by 2010 Task Force Criteria (TFC). Phenotype was ascertained using electrocardiograms, Holter monitoring, and cardiac imaging. Subjects were divided into “possible ARVC” (only genetic/familial predisposition) and “borderline ARVC” (one minor TFC criterion plus genetic/familial predisposition). We performed Cox regression to determine predictors, and multi-state modeling to assess probability of ARVC development. Results were replicated in an unrelated Italian cohort (57% male, 37.0 (IQR:25.4-50.4) years).

**Results:** At baseline, 93 (68%) had possible and 43 (32%) borderline ARVC. Follow-up was available for 123 (90%) relatives. After 8.1 (IQR:4.2-11.4) years, 41 (33%) developed definite ARVC. Independent of baseline phenotype, symptomatic subjects ( $p=0.014$ ) and those 20-30 years old ( $p=0.002$ ) had higher hazard of developing definite ARVC.

Furthermore, borderline ARVC patients had higher probability of developing definite ARVC compared to possible patients (1-year probability: 13% vs. 0.6%; 3-year probability: 35% vs. 5%,  $p<0.01$ ). External replication showed comparable results ( $p>0.05$ ).

**Conclusion:** Symptomatic relatives, those in 20-30 age range and with borderline ARVC have higher probability of developing definite ARVC. These patients may benefit from more frequent follow-up, while others may be monitored less often.

**Condensed abstract**

Relatives at risk for arrhythmogenic right ventricular cardiomyopathy (ARVC) are recommended to undergo frequent re-evaluations. An individualized approach may provide more efficient use of resources. Among 136 relatives, 33% progressed towards definite ARVC after 8.1 (IQR:4.2-11.4) years of follow-up. Symptomatic relatives ( $p=0.014$ ) and those 20-30 years old ( $p=0.002$ ) were at higher hazard of developing definite ARVC. Time to diagnosis significantly depended on baseline phenotype, with higher risk in borderline ARVC patients ( $p<0.01$ ). External replication showed comparable results ( $p>0.05$ ). Prioritizing relatives could have bi-directional effects: high-risk subjects can be adequately treated, while low-risk subjects may be less frequently followed.

**Keywords:** ARVC; family screening; predictors; screening interval; ventricular arrhythmia

**Abbreviations**

ARVC = Arrhythmogenic right ventricular cardiomyopathy

CI = Confidence interval

CMR = Cardiac magnetic resonance imaging

ECG = Electrocardiogram

HF = Heart failure

HR = Hazard Ratio

IQR = Interquartile range

PKP2 = Plakophilin-2

PLN = Phospholamban

TFC = Task Force Criteria

VA = Ventricular arrhythmias

Journal Pre-proof

## Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy predisposing patients to potentially life-threatening ventricular arrhythmias (VA) and sudden cardiac death.<sup>1</sup> A familial predisposition of the disease has been widely established. Cardiologists therefore not only need to care for affected patients, but also for a large number of unaffected relatives, of whom approximately one-third will develop definite ARVC.<sup>2</sup> Consequently, current guidelines recommend that relatives with a genetic or familial predisposition to develop ARVC are routinely evaluated every 1 to 3 years with at least electrocardiographic (ECG) recording, Holter monitoring and imaging, regardless of their clinical phenotype at first evaluation.<sup>3-5</sup> This results in a significant burden on clinical resources, as well as a large psychosocial impact on patients and their families.<sup>6</sup> The yield of this approach has however not been systematically evaluated.

Disease expression of ARVC is highly variable, even among those from the same family, or those carrying identical pathogenic variants. As such, a generalized approach to management and follow-up of at-risk relatives seems inappropriate and risk stratification for developing ARVC is desirable.<sup>2</sup> This is even more important, as potentially fatal consequences including sudden cardiac death may occur early in the disease course.<sup>7</sup> While these fatal events typically occur after definite ARVC diagnosis,<sup>8,9</sup> there may be limited time to intervene and a timely diagnosis can be lifesaving. As such, there is a need for substantive data to identify relatives at high-risk of developing ARVC.

The purpose of this study is to 1) determine predictors; and 2) assess probability of definite ARVC development over time stratified by baseline clinical characteristics. In order to do so, we leveraged a carefully genotyped and phenotyped cohort of at-risk ARVC relatives in the Netherlands ACM Registry, and subsequently replicated results in an unrelated Italian cohort.



## Methods

### *Study population*

This study is divided in two phases: analyses were first performed in the Netherlands ACM registry<sup>10</sup> as a “derivation cohort”, and subsequently replicated in an unrelated Italian “validation cohort”. From the Netherlands ACM registry ([www.acmregistry.nl](http://www.acmregistry.nl); UCC-UNRAVEL #12-387), we identified families managed at the University Medical Center Utrecht and Amsterdam University Medical Center in which the proband fulfilled definite ARVC diagnosis as per 2010 Task Force Criteria (TFC)<sup>11</sup> and had undergone comprehensive genetic testing for an ARVC-associated pathogenic variant. Among these families, all relatives who were eligible for cardiac screening based on current guidelines were identified (see Supplemental Methods). We only included relatives who did not fulfill definite TFC at time of first clinical evaluation and who had a complete ARVC evaluation at baseline, as described below. In addition, we restricted our inclusion to relatives who were  $\geq 14$  years of age, given the well-recognized difficulty of diagnosing ARVC in pediatric cohorts.<sup>12</sup> We subsequently replicated our results in an unrelated Italian cohort of ARVC relatives, with the same inclusion criteria as described above. This study followed the Code of Conduct and the Use of Data in Health Research and was approved by local ethics and/or institutional review boards.

### *Clinical evaluation*

Participants were evaluated as described previously.<sup>7,9</sup> The medical history of each relative was obtained by review of medical records, clinical evaluation, and patient interview. 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 special interest in ARVC. Relatives were divided based on their

relationship to the proband as first-, second- or third-degree relatives; first-degree relatives were additionally divided into parents, siblings, and children of the proband.

All subjects underwent guideline-recommended screening recommendation, which included a 12-lead ECG, a Holter monitor of at least 24 hours, and an imaging modality (echocardiogram or cardiac magnetic resonance imaging (CMR)).<sup>3-5</sup> Testing results from other modalities (e.g. electrophysiology studies or angiogram) were also collected. For follow-up evaluation, we included all available clinical testing performed at the discretion of the treating cardiologist, including 12-lead ECG, Holter monitoring of at least 24 hours and/or imaging (echocardiogram or CMR).

#### *ARVC diagnosis*

Diagnosis of ARVC was based on the 2010 TFC.<sup>11</sup> Within this framework, definite ARVC diagnosis is defined as fulfilling two major, one major plus two minor, or four minor criteria. By study design, all subjects fulfilled one major criterium in the family history category given their genetic or familial predisposition, and none fulfilled a definite ARVC diagnosis at first evaluation. As such, subjects were stratified by their baseline clinical phenotype: “possible ARVC” (i.e. only the major family history criterion) or “borderline ARVC” (i.e. fulfillment of 1 minor criterion plus the major family history criterion).

#### *Study outcomes*

The primary outcomes of this study were 1) development of a new TFC criterion during follow-up, that was absent at first evaluation; and 2) development of a definite ARVC diagnosis during follow-up as per 2010 TFC. Follow-up duration was calculated from the date of first evaluation to the date of reaching the endpoint or censoring, defined as the most recent follow-up at which the endpoints could be ascertained.

Secondary outcomes of this study were the occurrence of sustained VA and heart failure (HF) during follow-up. For the purpose of this study, sustained VA was defined as a

composite of sudden cardiac death, sudden cardiac arrest, spontaneous sustained ventricular tachycardia (VT)(VT lasting  $\geq 30$ s at  $\geq 100$ bpm and/or with hemodynamic compromise requiring cardioversion), ventricular fibrillation/flutter or appropriate implantable cardioverter defibrillator (ICD) intervention, as done previously.<sup>13</sup> HF was defined as stage C HF or worse, utilizing the American College of Cardiology/American Heart Association classification.<sup>14</sup>

### *Statistical analysis*

Nominal variables were expressed as number (%), and continuous variables as mean $\pm$ standard deviation or median (interquartile range (IQR)), as appropriate. Comparisons for binary variables were performed by Chi-square or Fisher's exact test. For continuous variables, independent t-test or Mann-Whitney U test were used. The overall probability of survival free from the respective endpoints was visualized using a Kaplan-Meier curve and compared using the log-rank test. Predictors for the primary endpoints were tested by Cox proportional hazard regression, and hazard ratios (HR) were reported with 95% confidence intervals (CI). The proportional hazard's assumption was checked for every predictor using Schoenfeld residuals. Given the particular age-related penetrance that has been observed in prior ARVC studies<sup>9</sup>, additional analyses were performed to evaluate the effect of age on the predefined endpoints.

Since this was a retrospective study without predefined follow-up dates, the date of diagnosis in our study population was dependent on the timing of outpatient clinic visits: i.e. our analyses were sensitive to detection bias. We therefore created a multi-state model to correct for this. In this model, we determined possible, borderline and definite ARVC as separate states from which an individual can transition to a more severe disease state. Transitioning to a less severe disease state (e.g. from borderline to possible ARVC) was deemed impossible. The multi-state model considers every outpatient clinic visit as an

observation at an arbitrary time (i.e. “snapshot”) in which it is unknown what the exact date of transitioning between disease states is: the model subsequently estimates the probability of transitioning to a more severe disease state between two timepoints. The resulting model was compared to guideline screening recommendations, by comparing non-definite ARVC and definite ARVC as states.

We repeated the multi-state model after excluding (i) pediatric relatives and (ii) subjects with the Phospholamban (*PLN*) p.Arg14del variant as a sensitivity analysis. In addition, the model was replicated in an independent cohort of Italian ARVC relatives as a validation effort.

A p-value<0.05 was considered statistically significant. Data was analyzed using R version 4.1.2 (Boston, MA, USA), including the survival, survminer and msm package.

## Results

### *Study population*

Our derivation cohort consisted of 136 patients from 66 families who were found not to fulfill ARVC diagnosis at complete baseline evaluation (Supplemental Figure 1). Baseline characteristics are shown in Table 1. Median age at first evaluation was 25.5 years (IQR:15.8-44.4 years), 62 (46%) were male, and 104 (77%) carried a (likely) pathogenic variant (most commonly Plakophilin-2 (*PKP2*); n=71/136, 52%). Almost all subjects were White with European ancestry (99.3%). The majority of relatives (n=91, 67%) were first-degree relatives to the proband. Overall, most relatives were asymptomatic and came to attention because of screening (n=102, 75%), while the remaining 34 (25%) relatives reported symptoms of which palpitations were described most frequently (n=18/136, 13%).

### *Baseline clinical evaluation*

At first clinical evaluation, 93 (68%) relatives were diagnosed with possible and 43 (32%) with borderline ARVC. Relatives with borderline ARVC were significantly older

compared to those with possible ARVC (37.1 (IQR:18.7-46.4) vs. 22.7 (IQR:15.2-43.4) years,  $p=0.029$ ) In addition, relatives with borderline ARVC were significantly more likely to carry a (likely) pathogenic variant as compared to those with possible ARVC ( $n=38/43$  (88%) vs.  $n=66/93$  (71%),  $p=0.030$ )(Table 1).

#### *Follow-up clinical evaluation*

Of 136 patients without definite ARVC diagnosis at first evaluation, 123 (90%) received at least one follow-up evaluation. There were no significant differences in baseline characteristics between those with and without follow-up (Supplemental Table 1).

All further analyses are restricted to the 123 relatives who underwent follow-up. These relatives were followed for a median of 8.1 (IQR:4.2-11.4) years. Disease trajectory for every relative is visualized in Supplemental Figure 2, whereas group summaries are shown in Figure 1.

Overall, 62 (50%) relatives developed a new TFC criterion. In those 62 relatives, median time to new TFC criterion was 4.3 (IQR:2.2-7.4) years (Figure 1A). New TFC criteria were most commonly observed on ECG ( $n=27/62$ , 44%), followed by Holter monitoring ( $n=20/62$ , 32%), and imaging ( $n=15/62$ , 24%). New imaging criteria were most commonly observed on CMR ( $n=9/15$ , 60%), followed by echocardiography ( $n=4/15$ , 27%) and both modalities at one timepoint ( $n=2/15$ , 13%).

In addition, 41 (33%) relatives progressed to definite ARVC. In those 41 relatives, median time to ARVC diagnosis was 4.7 (IQR:2.2-8.2) years (Figure 1B). Most ( $n=38/41$ , 93%) relatives who developed definite ARVC relied on ECG or Holter monitoring criteria for their diagnosis, whereas the remaining ( $n=3/41$ , 7%) reached diagnosis with solely imaging criteria.

#### *Association of baseline clinical phenotype with outcomes*

Figure 2 shows the timing of a newly developed TFC criterion (Figure 2A) and definite ARVC diagnosis (Figure 2B) stratified by possible and borderline ARVC at first evaluation. As can be appreciated, time to new TFC criterion was similar between those with possible and borderline ARVC at first evaluation ( $p=0.079$ ), suggesting that the rate of disease progression is comparable in relatives regardless of baseline clinical phenotype. In contrast, relatives with borderline ARVC at presentation progressed more rapidly to definite ARVC diagnosis as compared to relatives with possible ARVC at presentation (definite diagnosis reached after 3.6 (IQR:1.8-7.6) vs. 7.7 (IQR:3.9-8.7) years, respectively,  $p<0.001$ ).

Table 2A-B show predictors for development of the primary outcomes both univariable and after adjustment for baseline clinical phenotype. Subjects between 20-30 years of age showed a non-significant trend towards a higher hazard for both outcomes in univariable analysis (HR 1.81,  $p=0.088$  for new TFC development and HR 2.05,  $p=0.114$  for definite ARVC, compared to subjects  $<20$  years old), which became significant after adjustment for baseline clinical phenotype (HR 2.14,  $p=0.033$  for new TFC development and HR 4.64,  $p=0.002$  for definite ARVC). In addition, symptomatic subjects had higher hazard of developing definite ARVC, both in univariable (HR 2.17,  $p=0.016$ ) and multivariable (HR 2.21,  $p=0.014$ ) analysis.

#### *Clinical implications of guideline implementation*

We subsequently developed a multi-state model to determine the transition-time of baseline clinical phenotype to definite ARVC. Calibration plots showed that the model had an excellent fit between 0 to 8 years of follow-up (Supplemental Figure 3). While age and symptomatic status were significant predictors of developing definite ARVC as described above (Table 2A-B), these factors did not significantly improve model calibration (Supplemental Figure 4) and hence were excluded from the final model.

Figure 3A shows the fitted survival probability of our multi-state model. As can be appreciated, the 1-, 3-, and 5-year probability of progressing to definite ARVC diagnosis in the overall population was 6% (95% CI:4-8%), 16% (95% CI:12-23%) and 26% (95% CI:17-37%), respectively. In addition, patients with borderline ARVC had a >5-fold higher probability of progressing to definite ARVC compared to those with possible ARVC, which was consistent throughout the follow-up period (1-year probability:13% vs. 0.6%; 3-year probability:35% vs. 5%; 5-year probability:51% vs. 11%,  $p<0.01$ ).

Consequently, applying guideline recommendations for cardiac screening at 1 to 3 year intervals yielded significantly different results depending on baseline clinical phenotype (Figure 3B). For example, to obtain a comparable risk of the 1-year probability in relatives with borderline ARVC (1-year probability:13% (95% CI:10-18%)), the screening interval in those with possible ARVC can be delayed up to 5 years (5-year probability:11% (95% CI:8-16%)).

#### *Development of VA or HF during follow-up*

None of the 82 relatives without a definite ARVC diagnosis experienced a sustained VA or HF during follow-up. Therefore, we limited further analyses to the 41 relatives with a definite ARVC diagnosis. Of these relatives, 7 (17%) had an ICD implanted, and 8 (20%) received antiarrhythmic medication. Median duration of follow-up after ARVC diagnosis was 5.0 (IQR:2.6-7.0) years.

Overall, 2 (5%) relatives experienced a VA: 2 females (both *PKP2* carriers, 40 and 35 years old) experienced appropriate ICD intervention for monomorphic VT (cycle length 235ms and 285ms) 9.6 and 14.5 years after definite diagnosis, respectively. Furthermore, 2 (5%) relatives experienced HF: one female and a male (both *PLN* p.Arg14del variant carriers, 67 and 53 years old) were diagnosed with HF 3.1 and 3.5 years after definite diagnosis,

respectively. No relatives required hospitalization for HF. One (2%) relative died at 60 years of age from a non-cardiac cause (cancer).

### *Sensitivity analysis*

As a sensitivity analysis, we repeated the multi-state model after exclusion of (i) pediatric subjects (<18 years of age at first evaluation)(Supplemental Figure 5 for calibration); and (ii) patients with the *PLN* p.Arg14del variant (Supplemental Figure 6 for calibration). As can be appreciated from Supplemental Figure 7, this resulted in similar recommended screening intervals for possible and borderline ARVC patients with non-significant changes to the yield of screening between 0.5 and 6 years of follow-up( $p>0.05$ ).

### *Model replication in external validation cohort*

As a validation cohort, we included 49 Italian patients who fulfilled the inclusion criteria for our study. As shown in Supplemental Table 2, Italian patients were older (median 37.0 (IQR:25.4-50.4) vs. 25.4 (IQR:15.7-43.8) years,  $p=0.001$ ), more often first-degree relative to the proband (96% vs. 66%,  $p<0.001$ ) and more often had borderline ARVC at first evaluation (59% vs. 33%,  $p=0.002$ ). In addition, their genetic background was (expectedly) different with no *PLN* variant carriers (0% vs. 20%,  $p=0.002$ ) and a higher proportion of Desmoplakin variant carriers (33% vs. 2%,  $p<0.001$ ). Regardless, Figure 4 (Supplemental Figure 8 for calibration) showed similar yields of screening for possible and borderline ARVC patients between cohorts, which was not significant between 0.5 and 5 years of follow-up( $p>0.05$ ).

## **Discussion**

The genetic era has led to an increasing number of subjects at-risk for ARVC who come to clinical attention. Disease development of ARVC is however highly variable, and a one-size-fits-all approach to family screening and management may therefore be



inappropriate. To the best of our knowledge, this study is the first to scrutinize the yield and possible optimization of cardiac family screening recommendations in ARVC.

This study has several interesting results. First, we found that among relatives without definite ARVC diagnosis at first evaluation, time to development of a new TFC criterion is approximately 4.5 years, with a similar rate of progression in individuals with possible and borderline ARVC. Consequently, subjects with borderline ARVC at first evaluation reach ARVC diagnosis sooner than those with possible ARVC. Second, apart from baseline clinical evaluation, predictors of disease development include having symptoms and being 20-30 years of age at first evaluation. Third, adverse events including VA and HF are rare among relatives, and are only observed in those with definite ARVC in whom we observed an average 7.7 years delay between diagnosis and the event. Last, our multi-state model showed that screening recommendations may be adjusted depending on baseline clinical phenotype. This information may help clinicians taking care of these patients to use their clinical resources more efficiently, with the ultimate goal to deliver the right care to the right patient at the right time.

#### *Rates of disease progression*

The first important finding of our study is that, among relatives without a definite diagnosis at first evaluation, development of a new TFC criterion is relatively slow with approximately 4.5 years between first evaluation and development of a new TFC criterion. Similar to other studies<sup>9,15,16</sup>, we found that abnormalities are more frequently observed on ECG or Holter monitoring compared to imaging tests. As such, a focus on ECG and Holter monitoring over imaging tests may be justified. Moreover, stratifying between subjects with possible ARVC (i.e. those with a completely normal evaluation) and borderline ARVC (i.e. one minor abnormality in addition to their familial predisposition) yielded similar rates of disease progression. As a consequence, subjects with borderline ARVC reach definite ARVC

diagnosis sooner than subjects with possible ARVC. While this is an expected finding, it is important to recognize that this will impact yield of repeat screening depending on baseline clinical evaluation. Therefore, we believe this information should be taken into account when determining the optimal interval for repeat evaluation, as described below.

#### *Predictors of disease development*

Our study also explored clinical characteristics that are associated with ARVC development. We showed that age-related progression peaked in early adulthood with a 2-fold higher hazard for both endpoints in relatives 20-30 years of age compared to those <20 years of age. This confirms and extends findings from a previous study<sup>9</sup>, and will impact the recommended screening interval of relatives in this age category: subjects 20-30 years old are more likely to benefit from more frequent screening compared to those at either end of the age spectrum.

Additionally, our results showed that having symptoms at first evaluation is associated with a two-fold increased hazard of developing definite ARVC. Indeed, symptoms likely reflect (early) disease that may or may not be detected by clinical tests, as every clinical test is a “snapshot” in time and variability in test results occur. To our surprise and in contrast with a prior meta-analysis<sup>2</sup>, genotype did not predict disease development in our study. As per study design, we included all subjects who were eligible for guideline-recommended clinical ARVC family screening. This includes subjects with a known pathogenic ARVC-associated variant (i.e. being 100% at-risk), as well as first-degree relatives to a proband without a known pathogenic variant (i.e. being 50% at-risk, assuming an autosomal dominant Mendelian inheritance pattern). The fact that presence of a pathogenic variant did not predict disease development may have been a power issue, as the majority of our population (77%) carried a (likely) pathogenic variant.

#### *Rare adverse events*

The third important finding of our study is that VA and HF events are rare among relatives, and only occur among those who already have a definite ARVC diagnosis. Indeed, previous studies already suggested that phenotypic ARVC expression is a prerequisite for arrhythmic and HF events.<sup>8,9,16</sup> Our study confirms and extends these findings, by showing that all subjects with adverse events had a definite diagnosis, and determining a long time interval between diagnosis and those adverse events. Importantly, the latter constitutes the time to intervene in the disease course to prevent these adverse events, which is why re-evaluation is performed in the first place. It also confirms the importance of family screening: as long as relatives present themselves for screening at a cardiologist's practice, adverse events are unlikely to occur and may be prevented by adequate intervention. Moreover, a previous study<sup>16</sup>, reported only VA in relatives who had prior structural progression, which was also observed in both our relatives with VA. We are definitely aware of cases in the literature that presented with sudden cardiac death prior to clinical evaluation.<sup>17</sup> However, we believe the findings of this study are reassuring for clinicians and families, who need to live with the prospect of a disease that confers a lifelong risk of potentially fatal arrhythmic and HF events.

#### *Individualized approach to family screening*

Our study provides insights into the three key concepts of family screening: (1) who should be screened; (2) how often; and (3) by which methods. A visual representation of these concepts is shown in the Central Illustration.

(1) Who: Obviously, after diagnosis of ARVC in a proband, it is important that every at-risk relative undergoes a complete baseline evaluation (including 12-lead ECG, Holter monitoring, and imaging) to ascertain the presence or absence of disease. Indeed, this is a class I recommendation in the current guidelines<sup>3-5</sup> and was a prerequisite for inclusion in our study.

(2) How often: In case a definite ARVC diagnosis is not made at first evaluation, decisions should be made regarding screening intervals and tests to be performed during follow-up. While an acceptable risk threshold for developing definite ARVC is to be determined, our multi-state model can be used to determine the optimal screening interval in ARVC relatives. In our opinion, a screening interval of 1 year in borderline ARVC and 5 years in possible ARVC is justifiable: Figure 3B shows that these probabilities correspond to currently accepted screening recommendations for the overall population. Moreover, these probabilities were externally replicated with comparable results. While age and symptomatic status did not significantly add to our multi-state model, they were significant predictors of definite ARVC in Cox regression analysis. We would therefore err on the side of caution and re-evaluate symptomatic or young (20-30 years old) relatives with possible ARVC more frequently (e.g. at 1- to 2-year intervals), as to avoid missing any relevant disease progression in the interim.

(3) By which methods: In line with our study design, we would recommend that clinical evaluations include a 12-lead ECG, Holter monitoring and imaging modality. However, we and others have shown that disease progression is more often observed on ECG and Holter monitoring than on imaging tests.<sup>9,15,16</sup> This can be exploited when optimizing screening protocols: if it would be desirable for either the patient or physician to adopt a more frequent screening regimen, it might be prudent to focus on ECG and Holter monitoring, and only perform imaging in case abnormal findings are observed on these “electrical” tests.

It is important to recognize that, while this study provides guidance on the above-mentioned concepts, decisions regarding screening should always be made by shared decision-making and based on patient’ and physician preference. Of note, none of our

relatives experienced potentially fatal events prior to ARVC diagnosis, suggesting that patient safety is warranted by adopting our suggested approach.

### *Study limitations*

While our cohort of comprehensively evaluated (both in phenotype and in genotype) individuals is one of the largest cohorts of ARVC relatives to date, we were underpowered to perform extensive multivariable analyses to ascertain independent predictors of ARVC development. Also, it is important to recognize that previous studies have convincingly shown that exercise influences disease development in at-risk ARVC patients.<sup>18</sup> As such, the fact that exercise data was not available in our cohort is a limitation of our study. Since we routinely advise at-risk relatives to cease moderate to vigorous exercise as per current guidelines<sup>3</sup>, our screening recommendations should only be utilized in non-athletes. We excluded subjects without a complete baseline evaluation from this study, as we cannot guarantee that the endpoint was not already reached at baseline. While the performance of such a complete baseline evaluation may not have been at random, it is important to highlight that a complete baseline evaluation including 12-lead ECG, Holter monitoring, and imaging is prescribed at first evaluation in current ARVC guidelines. A recent meta-analysis<sup>2</sup> showed that relatives with a (likely) pathogenic variant had a higher prevalence of ARVC development and VA compared to relatives of gene-elusive families. Therefore, the genetic make-up of cohort with a large proportion of (likely) pathogenic variant carriers could have impacted our results. In addition, our results may have been influenced by the significant proportion of *PLN* variant carriers in our derivation cohort. Although both the sensitivity analyses and external replication were reassuring, we believe future studies should explore gene-specific clinical evaluation and follow-up in adequately powered multicenter cohorts.

### **Conclusion**

This study evaluated the predictors and probability of developing definite ARVC diagnosis in at-risk relatives during follow-up. We showed that disease development is slow, with a median time to new TFC development of approximately 4.5 years. Symptomatic patients and those between 20-30 years of age have higher hazard of developing definite ARVC. In addition, those with borderline ARVC are more likely to develop definite ARVC during medium-term follow-up. Importantly, adverse events including VA and HF are rare, and occur late after definite diagnosis. Therefore, symptomatic patients, those between 20-30 years of age, and those with borderline ARVC may benefit from more frequent follow-up, while others may be monitored less often.

**Perspectives**

Competency in Patient Care and Procedural Skills: Among relatives without a definite diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC) at first evaluation, progression toward development of a new diagnostic criterion occurs gradually (over approximately 5 years), and rates of progression are similar among those with possible and borderline ARVC.

Translational Outlook: Future studies should include more relatives of probands with ARVC to increase the statistical precision of individualized risk prediction and allow adjustment for high-risk baseline characteristics.

## References

1. Marcus FI, Fontaine GH, Guiraudon G, et al. Right ventricular dysplasia: A report of 24 adult cases. *Circulation*. 1982;65(2):384-398.
2. Sharma A, Bosman LP, Tichnell C, et al. Arrhythmogenic Right Ventricular Cardiomyopathy Prevalence and Arrhythmic Outcomes in At-Risk Family Members: A Systematic Review and Meta-Analysis. *Circ Genom Precis Med*. 2022;15(3):e003530.
3. Towbin JA, McKenna WJ, Abrams DJ, et al. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm*. 2019;16(11):e301-e372.
4. Corrado D, Wichter T, Link MS, et al. Treatment of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: An International Task Force Consensus Statement. *Circulation*. 2015;132(5):441-453.
5. Hershberger RE, Givertz MM, Ho CY, et al. Genetic Evaluation of Cardiomyopathy—A Heart Failure Society of America Practice Guideline. *J Card Fail*. 2018;24(5):281-302.
6. James CA, Tichnell C, Murray B, Daly A, Sears SF, Calkins H. General and disease-specific psychosocial adjustment in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy with implantable cardioverter defibrillators: a large cohort study. *Circ Cardiovasc Genet*. 2012;5(1):18-24.
7. Bhonsale A, Groeneweg JA, James CA, et al. Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. *Eur Heart J*. 2015;36(14):847-855.



8. Zorzi A, Rigato I, Pilichou K, et al. Phenotypic expression is a prerequisite for malignant arrhythmic events and sudden cardiac death in arrhythmogenic right ventricular cardiomyopathy. *Europace*. 2016;18(7):1086-1094.
9. te Riele ASJM, James CA, Groeneweg JA, et al. Approach to family screening in arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Eur Heart J*. 2016;37(9):755-763.
10. Bosman LP, Verstraelen TE, van Lint FHM, et al. The Netherlands Arrhythmogenic Cardiomyopathy Registry: design and status update. *Neth Heart J*. 2019;27(10):480-486.
11. Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Eur Heart J*. 2010;31(7):806-814.
12. Steinmetz M, Krause U, Lauerer P, et al. Diagnosing ARVC in Pediatric Patients Applying the Revised Task Force Criteria: Importance of Imaging, 12-Lead ECG, and Genetics. *Pediatr Cardiol*. 2018;39(6):1156-1164.
13. Cadrin-Tourigny J, Bosman LP, Nozza A, et al. A new prediction model for ventricular arrhythmias in arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J*. 2019;40(23):1850-1858.
14. Heidenreich PA, Bozkurt B, Aguilar D, et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2022 May 3;79(17):e263-e421.
15. Te Riele ASJM, James CA, Rastegar N, et al. The Yield of Serial Evaluation in At-Risk Family Members of Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy Patients Anneline. *J Am Coll Cardiol*. 2014;64(3):293-301.

16. Chivulescu M, Lie ØH, Popescu BA, et al. High penetrance and similar disease progression in probands and in family members with arrhythmogenic cardiomyopathy. *Eur Heart J*. 2020;41(14):1401-1410.
17. Isbister JC, Nowak N, Yeates L, et al. Concealed Cardiomyopathy in Autopsy-Inconclusive Cases of Sudden Cardiac Death and Implications for Families. *J Am Coll Cardiol*. 2022;80(22):2057-2068.
18. James C, Bhonsale A, Tichnell C, et al. Exercise increases age-related penetrance and arrhythmic risk in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated desmosomal mutation carriers. *J Am Coll Cardiol*. 2013;62(12):129-1297.

## Legends

### **Figure 1. Disease progression in the derivation cohort.**

(A) Survival curve of a new TFC criterion. (B) Survival curve of definite ARVC diagnosis during. Shaded areas indicate 95% CI. Abbreviations as in text.

### **Figure 2. Disease progression stratified by baseline clinical phenotype.**

Survival curve of a new TFC criterion (panel A) and definite ARVC diagnosis (panel B) in relatives with possible (yellow line) and borderline (blue line) ARVC diagnosis. Shaded areas indicate 95% CI. Abbreviations as in text.

### **Figure 3. The probability of developing definite ARVC during follow-up.**

(A) Fitted probability of developing definite ARVC. The probability of definite ARVC-free survival (Y-axis) is shown over time (X-axis) after first clinical evaluation. (B) Yield of screening with different screening intervals. Different screening intervals (X-axis) are shown against fitted probability of definite ARVC (Y-axis). The dotted black lines indicate the fitted risk of guideline-recommended screening intervals between 1-3 years. The overall population, possible ARVC and borderline ARVC are indicated by gray, yellow and blue lines/bars, respectively. Shaded areas and error bars indicate 95% CI. Abbreviations as in text.

### **Figure 4. Probability of definite ARVC in the derivation and validation cohorts.**

The fitted probability in those with possible (panel A) and borderline ARVC (panel B).

The fitted probability (i.e. yield of screening; Y-axis) is comparable in the derivation cohort (red bar) and validation cohort (blue bar) for a range of screening intervals between 0.5 and 5 years (X-axis). Error bars indicate 95% CI. Abbreviations as in text.

### **Central Illustration. Individualized approach to ARVC family screening.**

The three key concepts of family screening are graphically depicted. Who: all at-risk relatives\*. Importantly, relatives who are symptomatic or between 20-30 of age are at

increased risk of developing definite ARVC. How often: relatives with possible and borderline ARVC should be screened every 5 years and every year, respectively. Given the increased likelihood of developing definite ARVC, symptomatic subjects and those 20-30 years old should be screened every 1-2 years. By which methods: every evaluation should include a 12-lead ECG, Holter monitoring and an imaging modality. If more frequent screening is desired, a focus on 12-lead ECG and Holter monitoring is justified, with imaging tests employed only in the presence of abnormalities on these tests. \*As per guideline recommendations, relatives are considered at risk if they carry the same (likely) pathogenic variant as the proband and/or are first-degree relatives of the proband. Abbreviations as in text.

**Table 1.** Baseline characteristics.

	Overall (N=136)	Possible (N=93)	Borderline (N=43)	p-value
Age at presentation (years)	25.5 (15.8-44.4)	22.7 (15.2-43.4)	37.1 (18.7-46.4)	0.029
Age (categorical)				0.170
Younger than 20	54 (39.7)	41 (44.1)	13 (30.2)	
between 20 and 30	22 (16.2)	17 (18.3)	5 (11.6)	
between 30 and 40	14 (10.3)	8 (8.6)	6 (14.0)	
Older than 40	46 (33.8)	27 (29.0)	19 (44.2)	
Male sex	62 (45.6)	37 (39.8)	25 (58.1)	0.070
White with European ancestry	135 (99.3)	92 (98.9)	43 (100.0)	1.000
Relationship to proband				0.233
Child	55 (40.7)	43 (46.2)	12 (28.6)	
Parent	13 (9.6)	10 (10.8)	3 (7.1)	
Sibling	23 (17.0)	13 (14.0)	10 (23.8)	
2nd degree	26 (19.3)	16 (17.2)	10 (23.8)	
3rd degree or further	18 (13.3)	11 (11.8)	7 (16.7)	
(Likely) pathogenic variant	104 (76.5)	66 (71.0)	38 (88.4)	0.030
<i>PKP2</i>	71 (52.2)	44 (47.3)	27 (62.8)	0.101
<i>DSP</i>	2 (1.5)	1 (1.1)	1 (2.3)	0.534
<i>DSG2</i>	5 (3.7)	4 (4.3)	1 (2.3)	1.000
<i>PLN</i>	26 (19.1)	17 (18.3)	9 (20.9)	0.896
Symptoms at initial presentation				0.283
Asymptomatic	102 (75.0)	73 (78.5)	29 (67.4)	
Palpitations	18 (13.2)	10 (10.8)	8 (18.6)	
Pre-syncope	5 (3.7)	2 (2.2)	3 (7.0)	
Syncope	11 (8.1)	8 (8.6)	3 (7.0)	
ECG TFC fulfilment	32 (23.5)	0 (0.0)	32 (74.4)	<0.001
T wave inversion V1-2	4 (3.0)	0 (0.0)	4 (9.5)	0.008
T wave inversion V1-3	0 (0.0)	0 (0.0)	0 (0.0)	1.000
T wave inversion V4-6	4 (3.0)	0 (0.0)	4 (9.5)	0.008
T wave inversion with CRBBB V1-4	0 (0.0)	0 (0.0)	0 (0.0)	
Prolonged TAD	24 (17.6)	0 (0.0)	24 (55.8)	<0.001
Holter TFC fulfilment	9 (6.6)	0 (0.0)	9 (20.9)	<0.001
PVC count	2 (0-33)	2 (0-6)	19 (1-402)	<0.001
Imaging TFC fulfilment	3 (2.2)	0 (0.0)	3 (7.0)	0.030
CMR TFC fulfilment (N=67)	2 (3.0)	0 (0.0)	2 (9.5)	0.095
Presence of RV WMA	9 (13.4)	5 (11.9)	4 (16.0)	0.718
RVEDV/BSA (ml/m <sup>2</sup> )	91.9±21.0	91.7±14.2	92.4±31.4	0.905
RVEF (%)	54.0±7.4	55.2±6.3	51.7±9.1	0.086
LVEF (%)	57.9±6.4	59.1±4.2	55.5±8.9	0.036
Echocardiogram TFC fulfilment (N=114)	1 (0.9)	0 (0.0)	1 (2.6)	0.305
Presence of RV WMA	5 (4.5)	3 (3.8)	2 (6.1)	0.633
RVOT PLAX/BSA (mm/m <sup>2</sup> )	15.3±2.2	15.2±2.4	15.4±1.5	0.868
RVOT PSAX/BSA (mm/m <sup>2</sup> )	16.6±2.7	16.7±2.8	16.1±2.2	0.582
LVEF (%)	58.2±5.2	58.3±5.2	57.6±5.7	0.733

Variables are expressed as frequency (%), mean  $\pm$  standard deviation, or median (IQR). Total number of patients for a given variable are mentioned if missing data. Abbreviations: ARVC: Arrhythmogenic Right Ventricular Cardiomyopathy, BSA: Body Surface Area, CMR: Cardiac Magnetic Resonance, CRBBB: Complete right bundle Branch Block, *DSG2*: Desmoglein-2, *DSP*: Desmoplakin, ECG: electrocardiogram, LVEF: Left Ventricular Ejection Fraction *PKP2*: Plakophilin-2, PLAX: Parasternal Long Axis, *PLN*: Phospholamban, PSAX: Parasternal Short Axis, PVC: Premature Ventricular Complex, RVEDV: Right Ventricular End-Diastolic Volume, RVEF: Right Ventricular Ejection Fraction, RVOT: Right Ventricle Outflow Tract, TAD: Terminal Activation Duration, TFC: Task Force Criteria, WMA: Wall Motion Abnormalities.

**Table 2.** Cox proportional hazard regression for (A) new TFC criterion and (B) definite ARVC diagnosis.

**Table 2A**

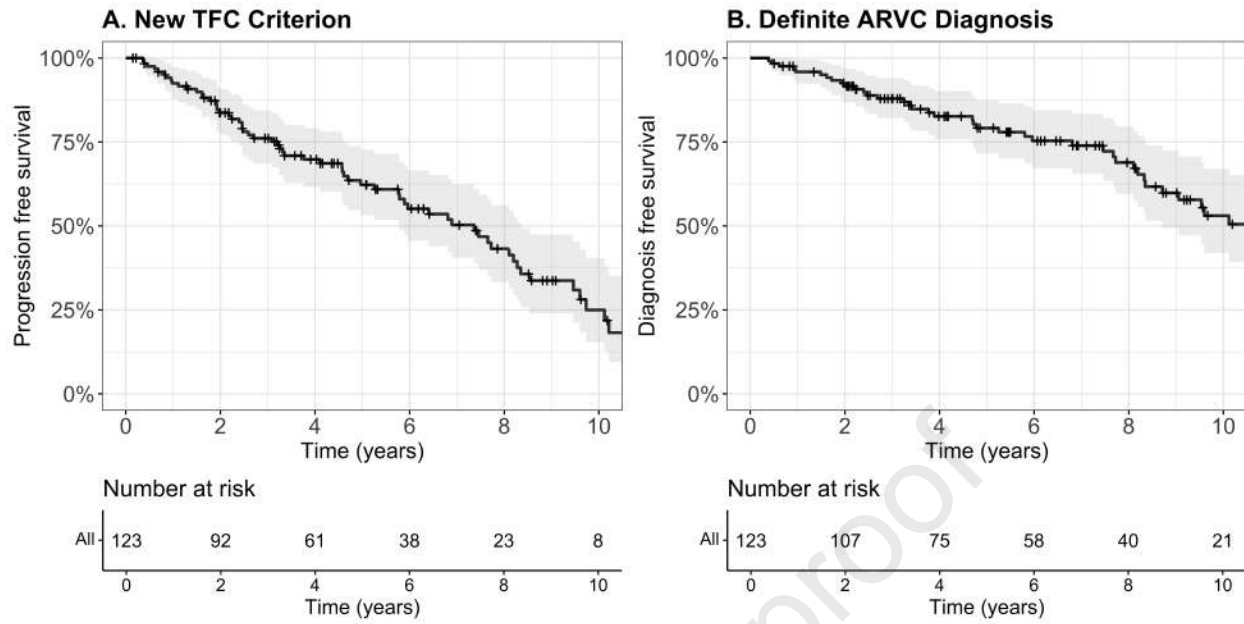
	Univariable analysis for new TFC criterion				Adjusted for baseline clinical phenotype			
	HR	Lower 95% CI	Upper 95% CI	p-value	HR	Lower 95% CI	Upper 95% CI	p-value
Age at presentation <sup>a</sup>								
20 – 30 years	1.81	0.92	3.56	0.088	2.14	1.06	4.32	0.033
30 – 40 years	1.53	0.68	3.47	0.305	1.35	0.59	3.09	0.473
> 40 years	0.84	0.44	1.58	0.579	0.78	0.41	1.47	0.440
Male sex	1.20	0.73	1.99	0.475	1.04	0.61	1.79	0.876
(Likely) pathogenic variant carrier	0.68	0.35	1.34	0.267	0.67	0.34	1.31	0.239
Sibling of the proband	1.58	0.85	2.94	0.146	1.51	0.81	2.82	0.191
Symptomatic	1.35	0.77	2.37	0.294	1.35	0.77	2.36	0.301

**Table 2B**

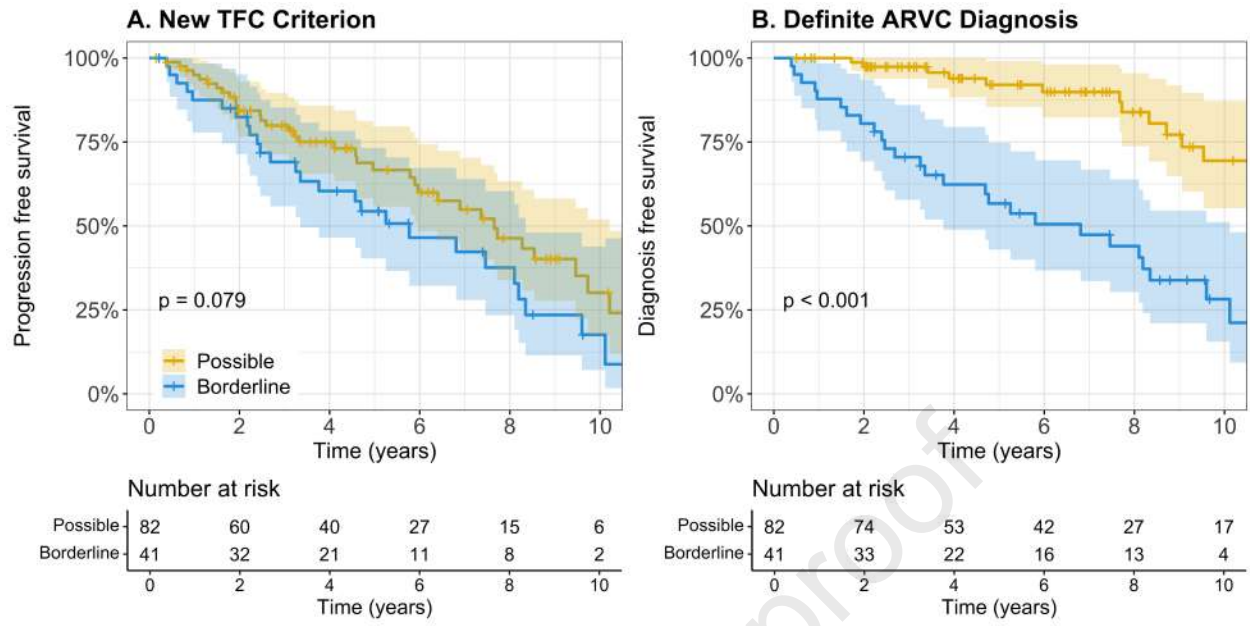
	Univariable analysis for definite ARVC diagnosis				Adjusted for baseline clinical phenotype			
	HR	Lower 95% CI	Upper 95% CI	p-value	HR	Lower 95% CI	Upper 95% CI	p-value
Age at presentation <sup>a</sup>								
20 – 30 years	2.05	0.84	4.96	0.114	4.64	1.73	12.49	0.002
30 – 40 years	1.51	0.57	3.97	0.410	1.35	0.51	3.58	0.548
> 40 years	1.51	0.71	3.22	0.284	1.23	0.57	2.65	0.594
Male sex	1.18	0.64	2.18	0.601	0.61	0.31	1.20	0.152
(Likely) pathogenic variant carrier	1.05	0.44	2.53	0.908	0.81	0.33	1.95	0.633
Sibling of the proband	1.37	0.65	2.88	0.407	1.39	0.66	2.94	0.385
Symptomatic	2.17	1.16	4.08	0.016	2.21	1.17	4.15	0.014

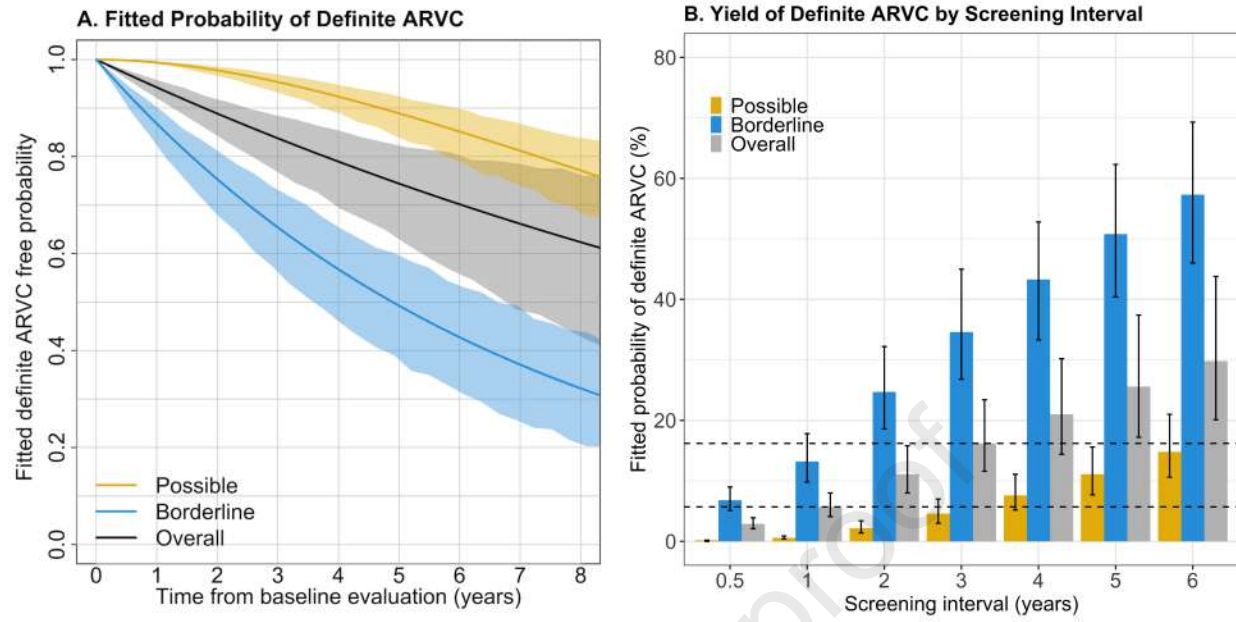
<sup>a</sup>Age subdivided into decades; all age subgroups are compared with the 14 – 20 age group.

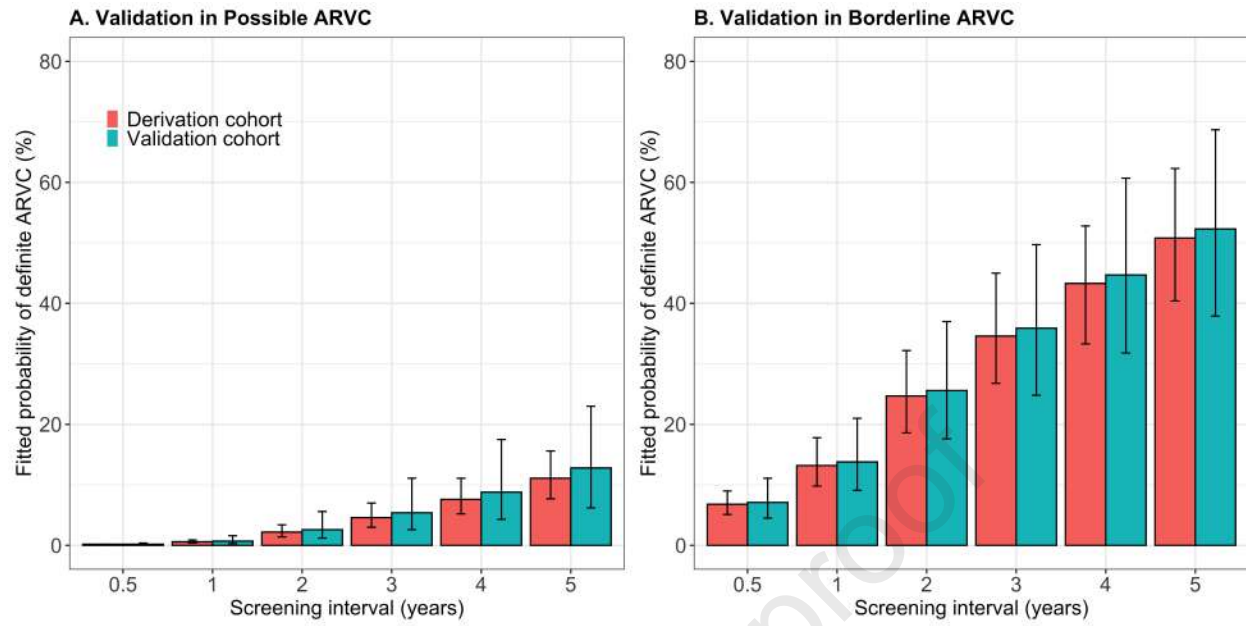
Abbreviations as in text.











## SUPPLEMENTAL APPENDIX

### Individualized Family Screening for Arrhythmogenic Right Ventricular Cardiomyopathy

#### Content

<b><i>Supplemental Methods</i> .....</b>	<b>2</b>
Eligibility for cardiac screening based on current guidelines.....	2
<b><i>Supplemental Tables</i> .....</b>	<b>3</b>
Supplemental Table 1. Baseline characteristics of relatives with and without follow-up in the Netherlands ACM registry. ....	3
Supplemental Table 2. Baseline characteristics of the derivation and validation cohort. ....	5
<b><i>Supplemental Figures</i> .....</b>	<b>7</b>
Supplemental Figure 1. Flowchart of the derivation cohort (Netherlands ACM registry). ....	7
Supplemental Figure 2. Disease progression in the derivation cohort.....	8
Supplemental Figure 3. Calibration slope of the multi-state model in the derivation cohort.....	10
Supplemental Figure 4. Calibration slope of the multi-state model including age and symptomatic status. ....	11
Supplemental Figure 5. Calibration slope of the multi-state model excluding pediatric cases (<18 years of age at first evaluation).....	12
Supplemental Figure 6. Calibration slope of the multi-state model excluding <i>PLN</i> pathogenic variant carriers.....	13
Supplemental Figure 7. Sensitivity analysis excluding pediatric cases (<18 years old at time of first evaluation) and <i>PLN</i> pathogenic variant carriers, separately.....	14
Supplemental Figure 8. Calibration slope of the multi-state model in the validation cohort. ....	15
<b><i>References</i>.....</b>	<b>16</b>

## Supplemental Methods

### Eligibility for cardiac screening based on current guidelines.

Among families with a proband carrying a (likely) pathogenic variant associated with ARVC, relatives were included if 1) they were genotyped and proved to carry the same genetic variant or 2) were not genotyped but were first-degree relatives of the proband; relatives were excluded if they did not harbor the familial genetic variant. Among families where there was no (likely) pathogenic variant identified in the proband, all first-degree relatives were included.<sup>1-3</sup> A tabulated overview of whom was eligible for inclusion is displayed below.

	Proband with LP/P variant	Proband without LP/P variant
Relative with same LP/P variant	Included in study	Not applicable
Relative not harboring the same LP/P variant	Excluded from study	Not applicable
First-degree relative who did not undergo genetic testing	Included in study	Included in study
Second-degree (or further) relative who did not undergo genetic testing	Excluded from study	Excluded from study

Abbreviations: LP: Likely pathogenic; P: Pathogenic.

### Supplemental Tables

**Supplemental Table 1.** Baseline characteristics of relatives with and without follow-up in the Netherlands ACM registry.

	Overall (N=136)	Follow-up (N=123)	No follow-up (N=13)	p-value
Age at presentation (years)	25.5 (15.8-44.4)	25.4 (15.7-43.8)	29.4 (18.1-51.5)	0.471
Male sex	62 (45.6)	55 (44.7)	7 (53.8)	0.737
White with European ancestry	135 (99.3)	122 (99.2)	13 (100.0)	1.000
Relationship to proband				0.072
Child	55 (40.7)	50 (41.0)	5 (38.5)	
Parent	13 (9.6)	9 (7.4)	4 (30.8)	
Sibling	23 (17.0)	22 (18.0)	1 (7.7)	
2nd degree	26 (19.3)	23 (18.9)	3 (23.1)	
3rd degree or further	18 (13.3)	18 (14.8)	0 (0.0)	
(Likely) pathogenic variant	104 (76.5)	96 (78.0)	8 (61.5)	0.185
<i>PKP2</i>	71 (52.2)	65 (52.8)	6 (46.2)	0.773
<i>DSP</i>	2 (1.5)	2 (1.6)	0 (0.0)	1.000
<i>DSG2</i>	5 (3.7)	4 (3.3)	1 (7.7)	0.400
<i>PLN</i>	26 (19.1)	25 (20.3)	1 (7.7)	0.465
Symptoms at initial presentation				0.532
Asymptomatic	102 (75.0)	93 (75.6)	9 (69.2)	
Palpitations	18 (13.2)	15 (12.2)	3 (23.1)	
Pre-syncope	5 (3.7)	5 (4.1)	0 (0.0)	
Syncope	11 (8.1)	10 (8.1)	1 (7.7)	
ECG TFC fulfilment	32 (23.5)	30 (24.4)	2 (15.4)	0.732
T wave inversion V1-2	4 (3.0)	3 (2.5)	1 (7.7)	0.336
T wave inversion V1-3	0 (0.0)	0 (0.0)	0 (0.0)	1.000
T wave inversion V4-6	4 (3.0)	4 (3.3)	0 (0.0)	1.000
T wave inversion with CRBBB V1-4	0 (0.0)	0 (0.0)	0 (0.0)	
Prolonged TAD	24 (17.6)	23 (18.7)	1 (7.7)	0.464
Holter TFC fulfilment	9 (6.6)	9 (7.3)	0 (0.0)	0.600
PVC count	2 (0-33)	2 (0-45)	8 (0-25)	0.774
Imaging TFC fulfilment	3 (2.2)	3 (2.4)	0 (0.0)	1.000
CMR TFC fulfilment (N=67)	2 (3.0)	2 (3.3)	0 (0.0)	1.000
Presence of RV WMA	9 (13.4)	9 (14.3)	0 (0.0)	1.000
RVEDV/BSA (ml/m <sup>2</sup> )	91.9±21.0	91.8±21.7	93.5±7.8	0.878
RVEF (%)	54.0±7.4	54.1±7.5	53.2±8.1	0.823
LVEF (%)	57.9±6.4	57.8±6.5	59.5±4.38	0.612
Echocardiogram TFC fulfilment (N=114)	1 (0.9)	1 (0.9)	0 (0.0)	1.000
Presence of RV WMA	5 (4.5)	5 (4.9)	0 (0.0)	1.000
RVOT PLAX/BSA (mm/m <sup>2</sup> )	15.3±2.2	15.2±2.2	15.4±1.8	0.873
RVOT PSAX/BSA (mm/m <sup>2</sup> )	16.6±2.7	16.6±2.8	16.9±0.6	0.791
LVEF (%)	58.2±5.2	58.1±5.2	58.4±7.3	0.925
Possible ARVC	93 (68.4)	82 (66.7)	11 (84.6)	0.313

Variables are expressed as frequency (%), mean  $\pm$  standard deviation, or median (IQR) as appropriate. Total number of patients for a given variable are mentioned if there were missing data. Abbreviations: ARVC: Arrhythmogenic Right Ventricular Cardiomyopathy, BSA: Body Surface Area, CMR: Cardiac Magnetic Resonance, CRBBB: Complete right bundle Branch Block, *DSG2*: Desmoglein-2, *DSP*: Desmoplakin, ECG: electrocardiogram, LVEF: Left Ventricular Ejection Fraction, *PKP2*: Plakophilin-2, PLAX: Parasternal Long Axis, *PLN*: Phospholamban, PSAX: Parasternal Short Axis, PVC: Premature Ventricular Complex, RVEDV: Right Ventricular End-Diastolic Volume, RVEF: Right Ventricular Ejection Fraction, RVOT: Right Ventricle Outflow Tract, TAD: Terminal Activation Duration, TFC: Task Force Criteria, WMA: Wall Motion Abnormalities.

**Supplemental Table 2.** Baseline characteristics of the derivation and validation cohort.

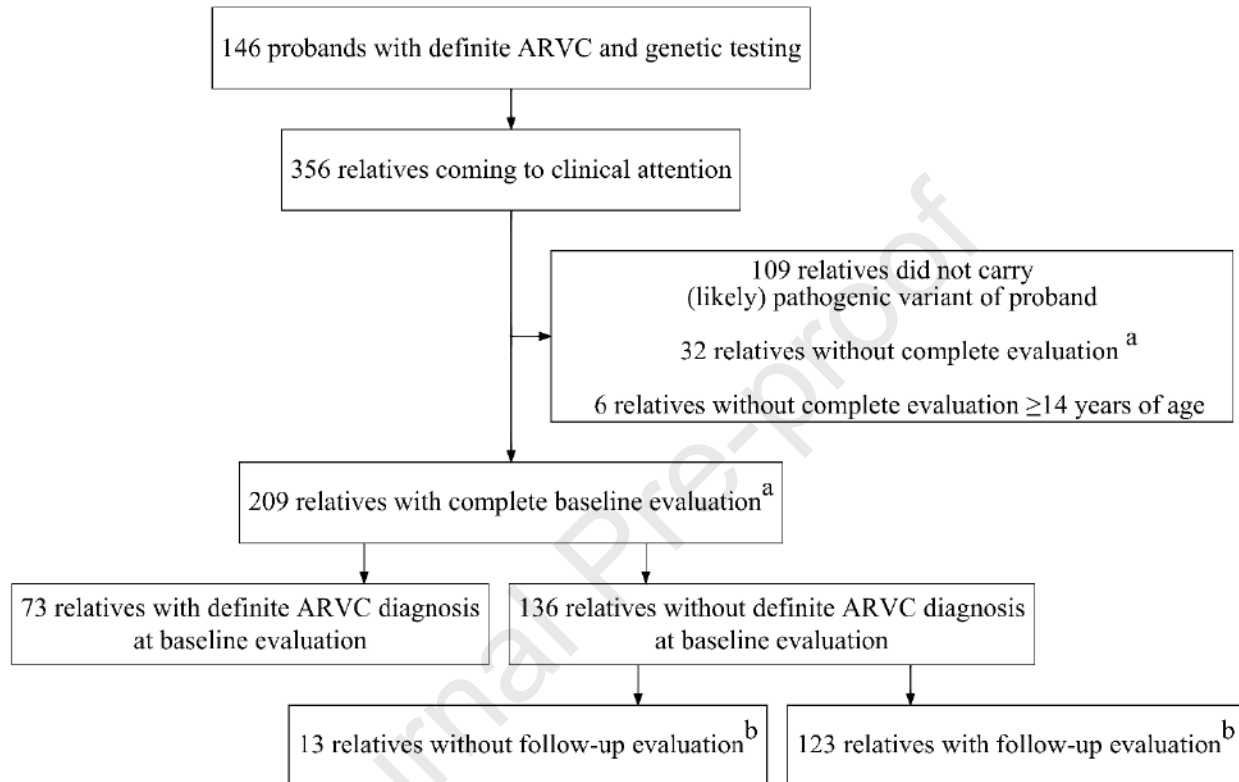
	Overall (N=172) <sup>a</sup>	Derivation (N=123) <sup>a</sup>	Validation (N=49) <sup>a</sup>	p-value
Age at presentation (years)	29.8 (18.7-44.8)	25.4 (15.7-43.8)	37.0 (25.4-50.4)	0.001
Male sex	83 (48.3)	55 (44.7)	28 (57.1)	0.193
White with European ancestry	170 (98.8)	122 (99.2)	48 (98.0)	0.233
Relationship to proband				<0.001
Child	70 (40.9)	50 (41.0)	20 (40.8)	
Parent	23 (13.5)	9 (7.4)	14 (28.6)	
Sibling	35 (20.5)	22 (18.0)	13 (26.5)	
2nd degree	25 (14.6)	23 (18.9)	2 (4.1)	
3rd degree or further	18 (10.5)	18 (14.8)	0 (0.0)	
(Likely) pathogenic variant	129 (75.0)	96 (78.0)	33 (67.3)	0.173
<i>PKP2</i>	74 (43.0)	65 (52.8)	9 (18.4)	<0.001
<i>DSP</i>	18 (10.5)	2 (1.6)	16 (32.7)	<0.001
<i>DSG2</i>	6 (3.5)	4 (3.3)	2 (4.1)	1.000
<i>DSC2</i>	1 (0.6)	0 (0.0)	1 (2.0)	0.633
<i>TMEM43</i>	3 (1.7)	0 (0.0)	3 (6.1)	0.034
<i>DES</i>	2 (1.2)	0 (0.0)	2 (4.1)	0.143
<i>PLN</i>	25 (14.5)	25 (20.3)	0 (0.0)	0.002
Symptoms at initial presentation	47 (27.3)	30 (24.4)	17 (34.7)	0.171
ECG TFC fulfilment	41 (23.8)	30 (24.4)	11 (22.4)	0.845
T wave inversion V1-2	8 (4.7)	3 (2.5)	5 (10.2)	0.044
T wave inversion V1-3	0 (0.0)	0 (0.0)	0 (0.0)	1.000
T wave inversion V4-6	9 (5.3)	4 (3.3)	5 (10.2)	0.121
T wave inversion with CRBBB V1-4	0 (0.0)	0 (0.0)	0 (0.0)	
Prolonged TAD	24 (14.0)	23 (18.7)	1 (2.0)	0.009
Holter TFC fulfilment	26 (15.2)	9 (7.3)	17 (35.4)	<0.001
PVC count	6 (1-270)	2 (0-45)	330 (30-1763)	<0.001
Imaging TFC fulfilment	3 (1.7)	3 (2.4)	0 (0.0)	0.559
CMR TFC fulfilment (N=94)	2 (1.9)	2 (3.3)	0 (0.0)	0.507
Presence of RV WMA	10 (9.5)	9 (14.3)	1 (2.4)	0.048
RVEDV/BSA (ml/m <sup>2</sup> )	87.9±19.22	91.8±21.7	82.7±13.9	0.021
RVEF (%)	53.8±6.8	54.1±7.5	53.4±5.9	0.625
LVEF (%)	56.8±6.9	57.8±6.5	55.5±7.2	0.097
Echocardiogram TFC fulfilment (N=137)	1 (0.6)	1 (0.9)	0 (0.0)	1.000
Presence of RV WMA	5 (3.4)	5 (4.9)	0 (0.0)	0.324
RVOT PLAX/BSA (mm/m <sup>2</sup> )	15.3±2.3	15.2±2.2	15.7±2.4	0.568
RVOT PSAX/BSA (mm/m <sup>2</sup> )	16.5±3.0	16.6±2.8	16.4±3.6	0.922
LVEF (%)	57.0±6.9	58.1±5.2	56.1±7.8	0.212
Possible ARVC	102 (59.3)	82 (66.7)	20 (40.8)	0.002
Definite ARVC during follow-up	60 (34.9)	42 (34.1)	18 (36.7)	0.885



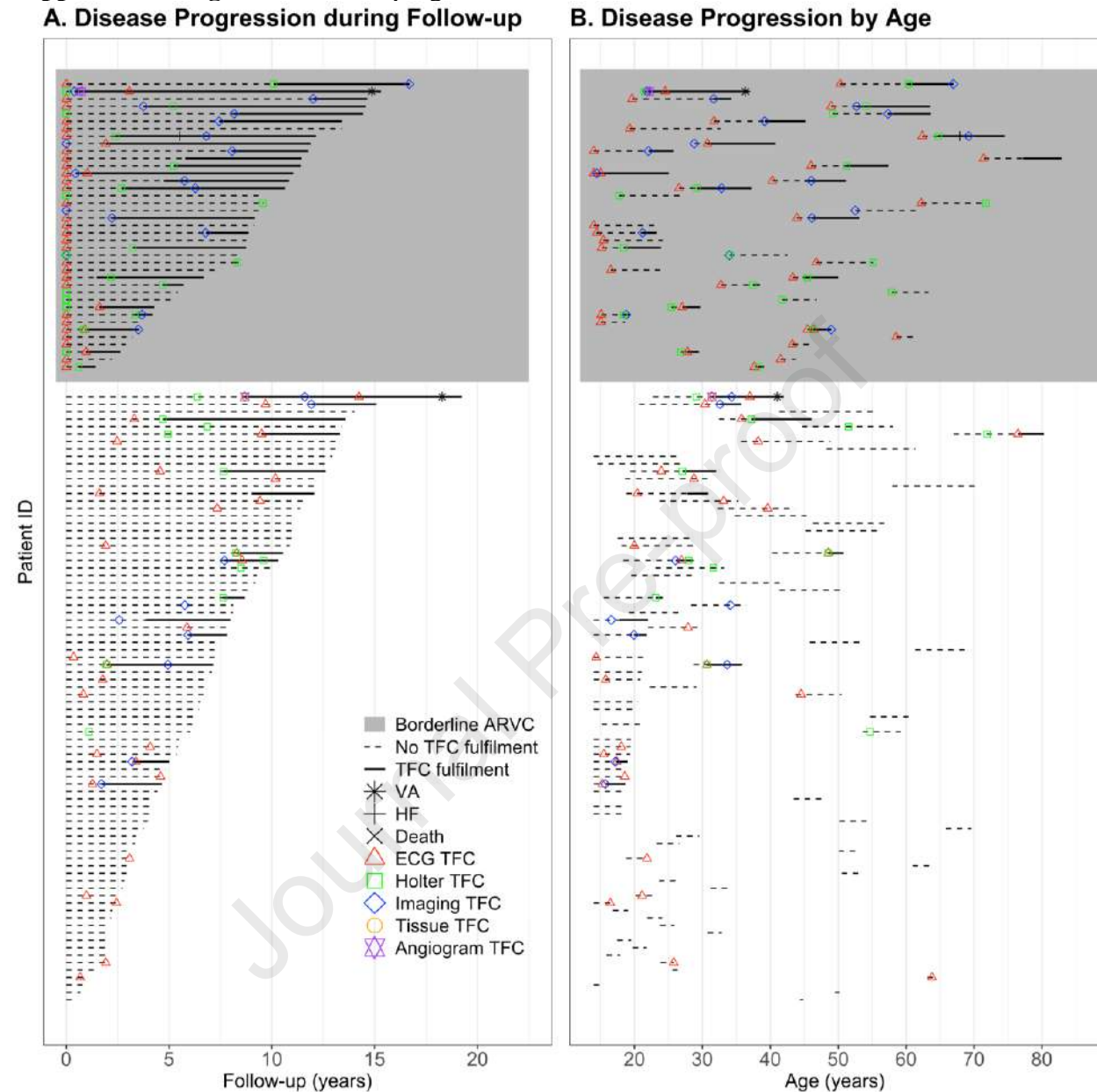
<sup>a</sup>Comparisons were made between 123 subjects with follow-up in the derivation cohort and 49 subjects with follow-up in the derivation cohort: the remaining 13 relatives without follow-up in the derivation cohort were disregarded as their absence of follow-up precluded them from inclusion in the multi-state model. Variables are expressed as frequency (%), mean  $\pm$  standard deviation, or median (IQR). Total number of patients for a given variable mentioned if missing data. Abbreviations: ARVC: Arrhythmogenic Right Ventricular Cardiomyopathy, BSA: Body Surface Area, CMR: Cardiac Magnetic Resonance, CRBBB: Complete right bundle Branch Block, *DES*: Desmin, *DSC2*: Desmocollin-2, *DSG2*: Desmoglein-2, *DSP*: Desmoplakin, ECG: electrocardiogram, LVEF: Left Ventricular Ejection Fraction, *PKP2*: Plakophilin-2, PLAX: Parasternal Long Axis, *PLN*: Phospholamban, PSAX: Parasternal Short Axis, PVC: Premature Ventricular Complex, RVEDV: Right Ventricular End-Diastolic Volume, RVEF: Right Ventricular Ejection Fraction, RVOT: Right Ventricle Outflow Tract, TAD: Terminal Activation Duration, TFC: Task Force Criteria, *TMEM43*: Transmembrane protein 43, WMA: Wall Motion Abnormalities.

### Supplemental Figures

**Supplemental Figure 1.** Flowchart of the derivation cohort (Netherlands ACM registry).



<sup>a</sup>“Complete baseline evaluation” defined as at least 12-lead electrocardiogram, Holter monitoring and imaging (cardiac magnetic resonance and/or echocardiography). <sup>b</sup>“Follow-up evaluation” defined as at least one of the tests listed above. Abbreviations: ARVC: Arrhythmogenic Right Ventricular Cardiomyopathy.

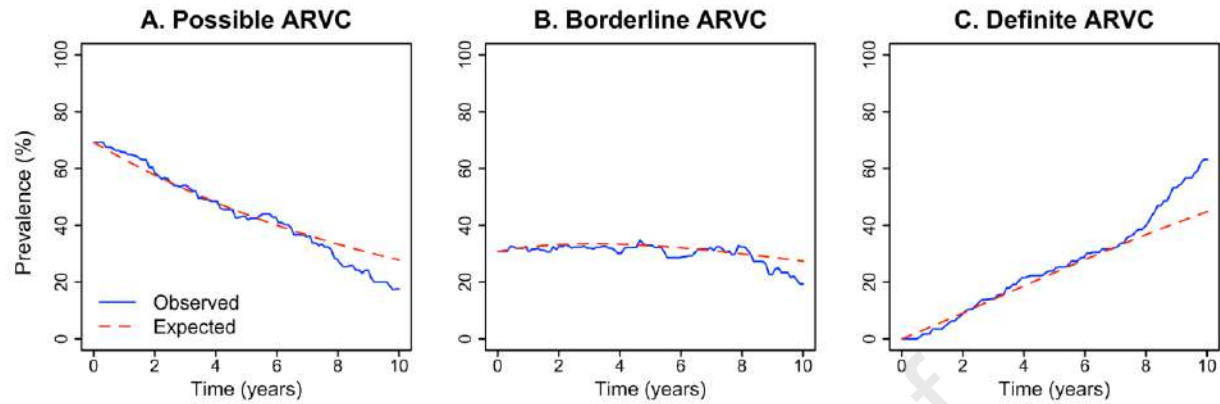
**Supplemental Figure 2.** Disease progression in the derivation cohort.

Clinical course of all relatives included in the derivation cohort. (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 possible ARVC 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), orange circle (tissue) and purple star (angiogram) indicate new TFC of the respective diagnostic test during follow-up. An asterisk, plus sign and multiplication sign visualize the occurrence of sustained VA, HF, and death, respectively.

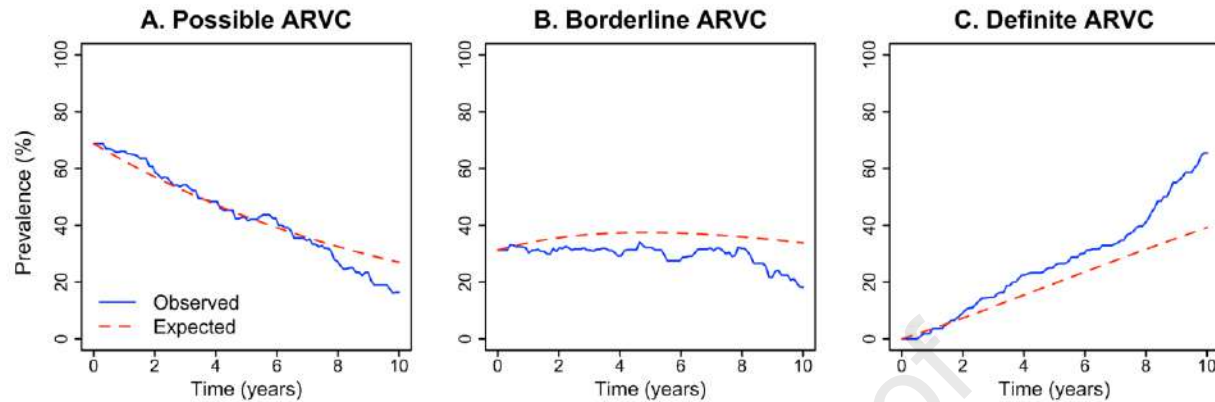
Abbreviations: ARVC: Arrhythmogenic Right Ventricular Cardiomyopathy, ECG:

Electrocardiogram, HF: Heart Failure, TFC: Task Force Criteria, VA: Ventricular Arrhythmia.

**Supplemental Figure 3.** Calibration slope of the multi-state model in the derivation cohort.

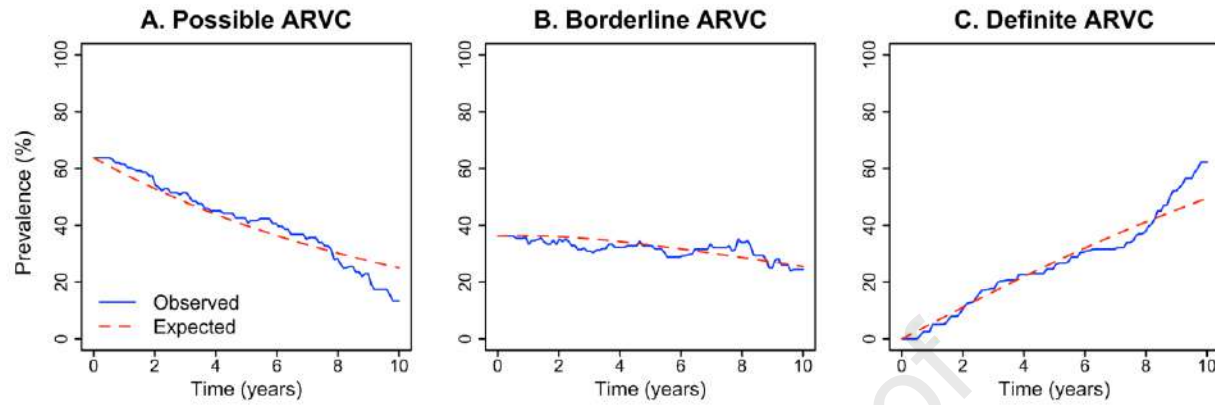
The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse of the definite ARVC prevalence plot. Abbreviations as in text.

**Supplemental Figure 4.** Calibration slope of the multi-state model including age and symptomatic status.



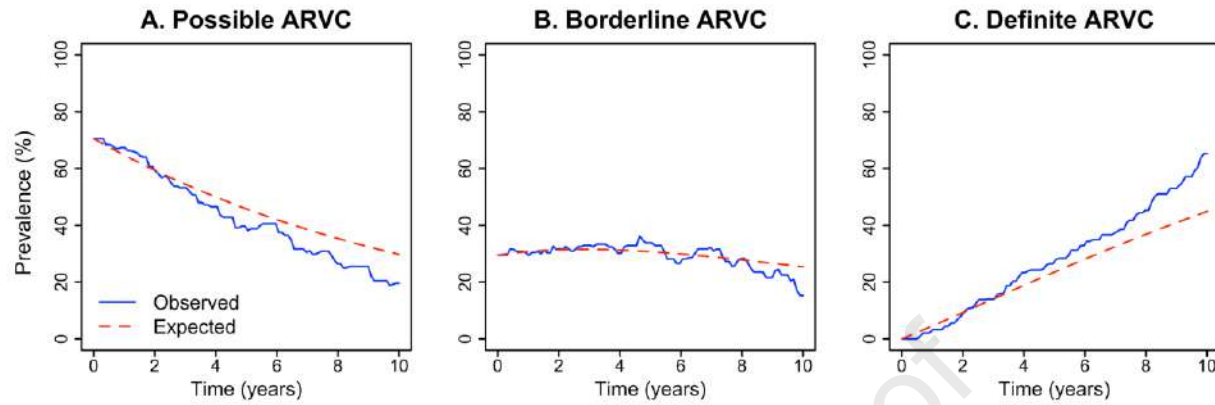
The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse of the definite ARVC prevalence plot. Abbreviations as in text.

**Supplemental Figure 5.** Calibration slope of the multi-state model excluding pediatric cases (<18 years of age at first evaluation).



The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse of the definite ARVC prevalence plot. Abbreviations as in text.

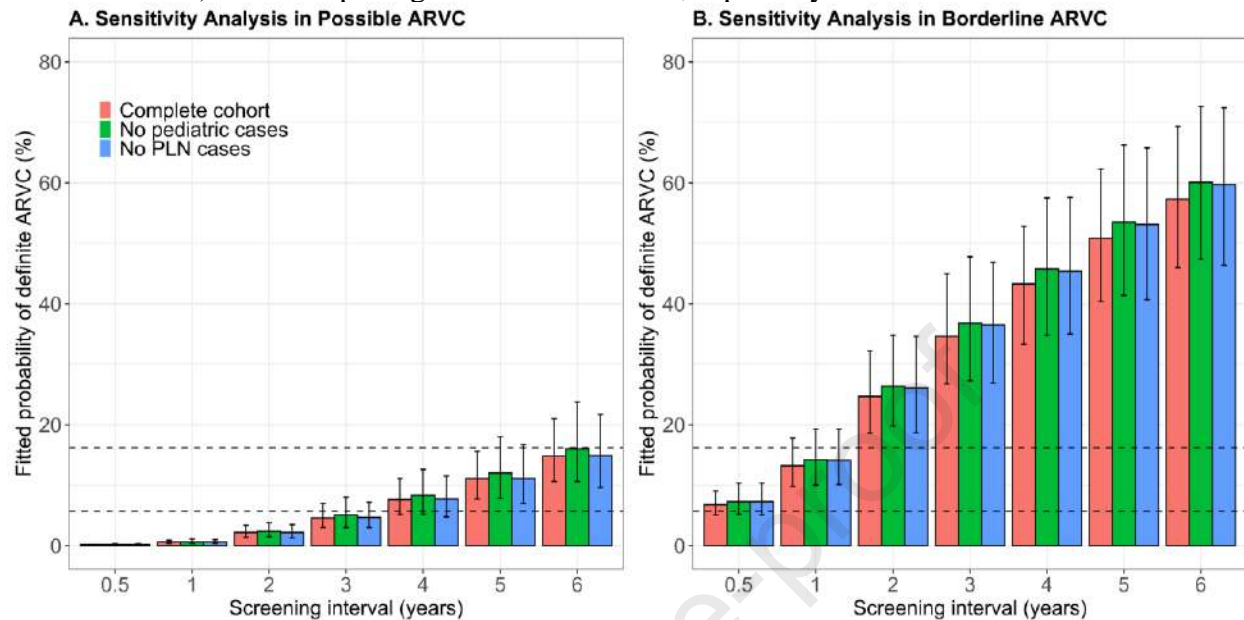
**Supplemental Figure 6.** Calibration slope of the multi-state model excluding *PLN* pathogenic variant carriers.



The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse of the definite ARVC prevalence plot. Abbreviations as in text.

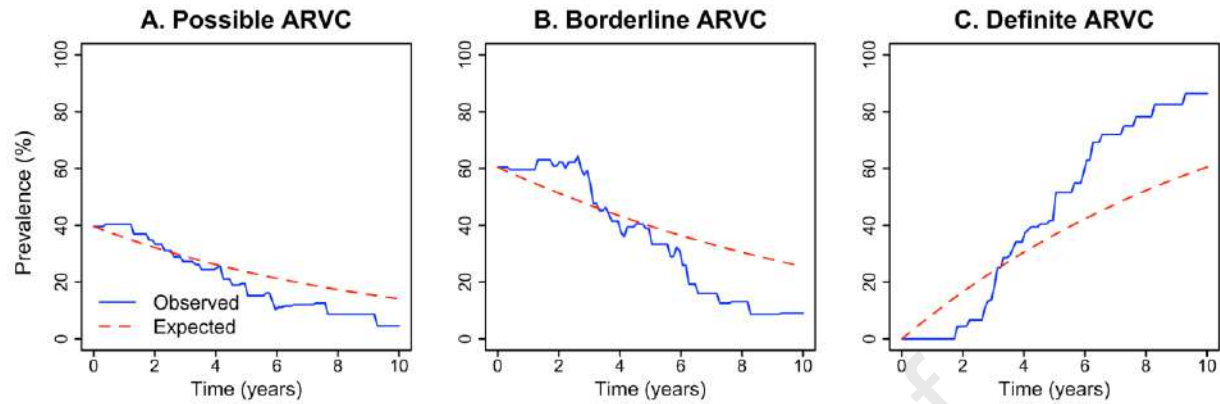


**Supplemental Figure 7.** Sensitivity analysis excluding pediatric cases (<18 years old at time of first evaluation) and *PLN* pathogenic variant carriers, separately.



As a sensitivity analysis, the multi-state model was repeated after exclusion of (i) pediatric subjects (<18 of age at baseline)(green, center bar); and (ii) patients with the founder variant in *PLN* (p.Arg14del)(blue, right bar). Both multi-state models were subsequently compared to the multi-state model of complete cohort (red, left bar). Different screening intervals (X-axis) are shown against the fitted probability of transitioning towards definite ARVC (Y-axis). The fitted probability in possible and borderline ARVC patients are visualized in panel A and B, respectively. The error bars indicate 95% CI and the dotted black lines indicate the fitted probability of the guideline-recommended screening interval of 1 and 3 years in the overall population. Using the complete cohort as a gold standard, the fitted probability of both “No pediatric cases” as well as “No *PLN*” cases” showed similar results for possible and borderline ARVC patients between 0.5 and 6 years of follow-up.

**Supplemental Figure 8.** Calibration slope of the multi-state model in the validation cohort.



The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse of the definite ARVC prevalence plot. Abbreviations as in text.

**References**

1. Towbin JA, McKenna WJ, Abrams DJ, et al. 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm*. 2019;16(11):e301-e372.
2. Corrado D, Wichter T, Link MS, et al. Treatment of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: An International Task Force Consensus Statement. *Circulation*. 2015;132(5):441-453.
3. Hershberger RE, Givertz MM, Ho CY, et al. Genetic Evaluation of Cardiomyopathy—A Heart Failure Society of America Practice Guideline. *J Card Fail*. 2018;24(5):281-302.