SUPPLEMENTAL APPENDIX

Individualized Family Screening for Arrhythmogenic Right Ventricular Cardiomyopathy

Content

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.[1–3] 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
PKP2	71 (52.2)	65 (52.8)	6 (46.2)	0.773
DSP	2 (1.5)	2 (1.6)	0 (0.0)	1.000
DSG2	5 (3.7)	4 (3.3)	1 (7.7)	0.400
PLN	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^2)	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^2)	15.3±2.2	15.2±2.2	15.4±1.8	0.873
RVOT PSAX/BSA (mm/m^2)	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 ± 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.

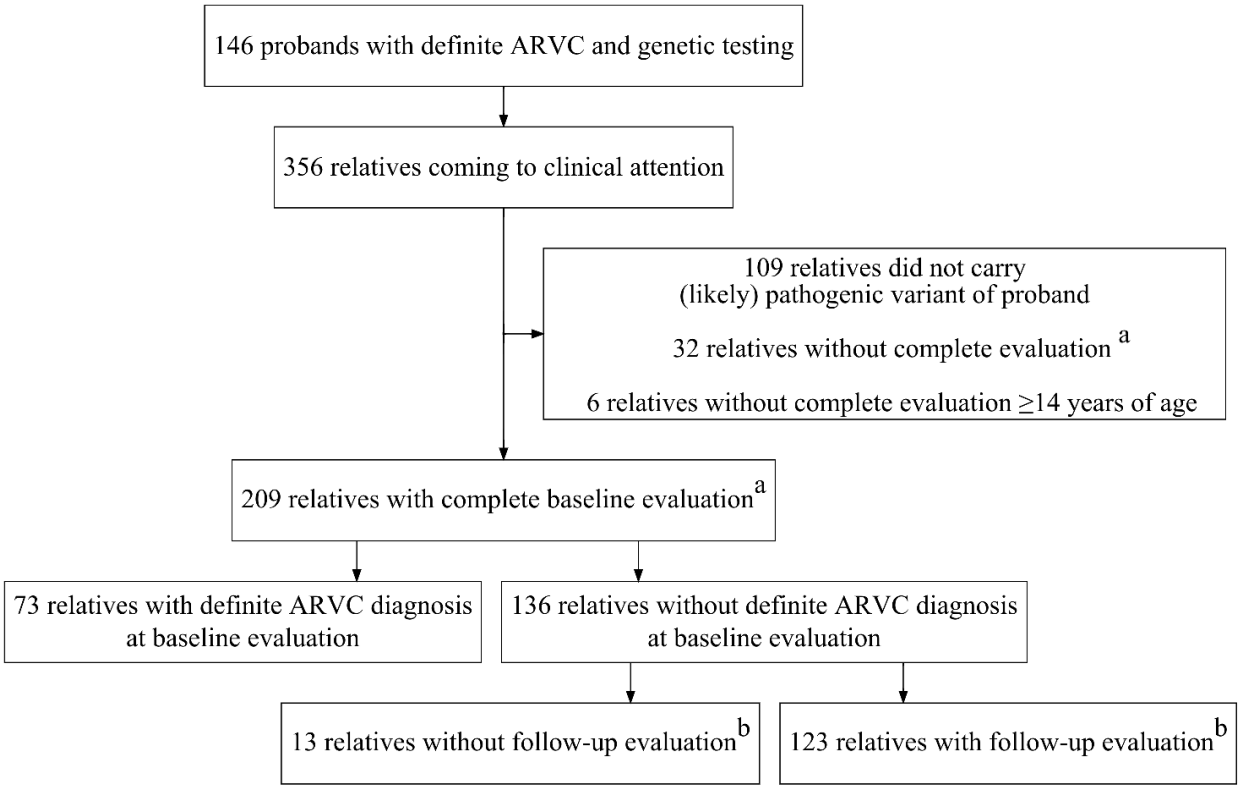
Supplemental Table 2. Baseline characteristics of the derivation and validation cohort.

	Overall (N=172)[a]	Derivation (N=123)[a]	Validation (N=49)[a]	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
PKP2	74 (43.0)	65 (52.8)	9 (18.4)	<0.001
DSP	18 (10.5)	2 (1.6)	16 (32.7)	<0.001
DSG2	6 (3.5)	4 (3.3)	2 (4.1)	1.000
DSC2	1 (0.6)	0 (0.0)	1 (2.0)	0.633
TMEM43	3 (1.7)	0 (0.0)	3 (6.1)	0.034
DES	2 (1.2)	0 (0.0)	2 (4.1)	0.143
PLN	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^2)	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^2)	15.3±2.3	15.2±2.2	15.7±2.4	0.568
RVOT PSAX/BSA (mm/m^2)	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

[a]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 ± 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.

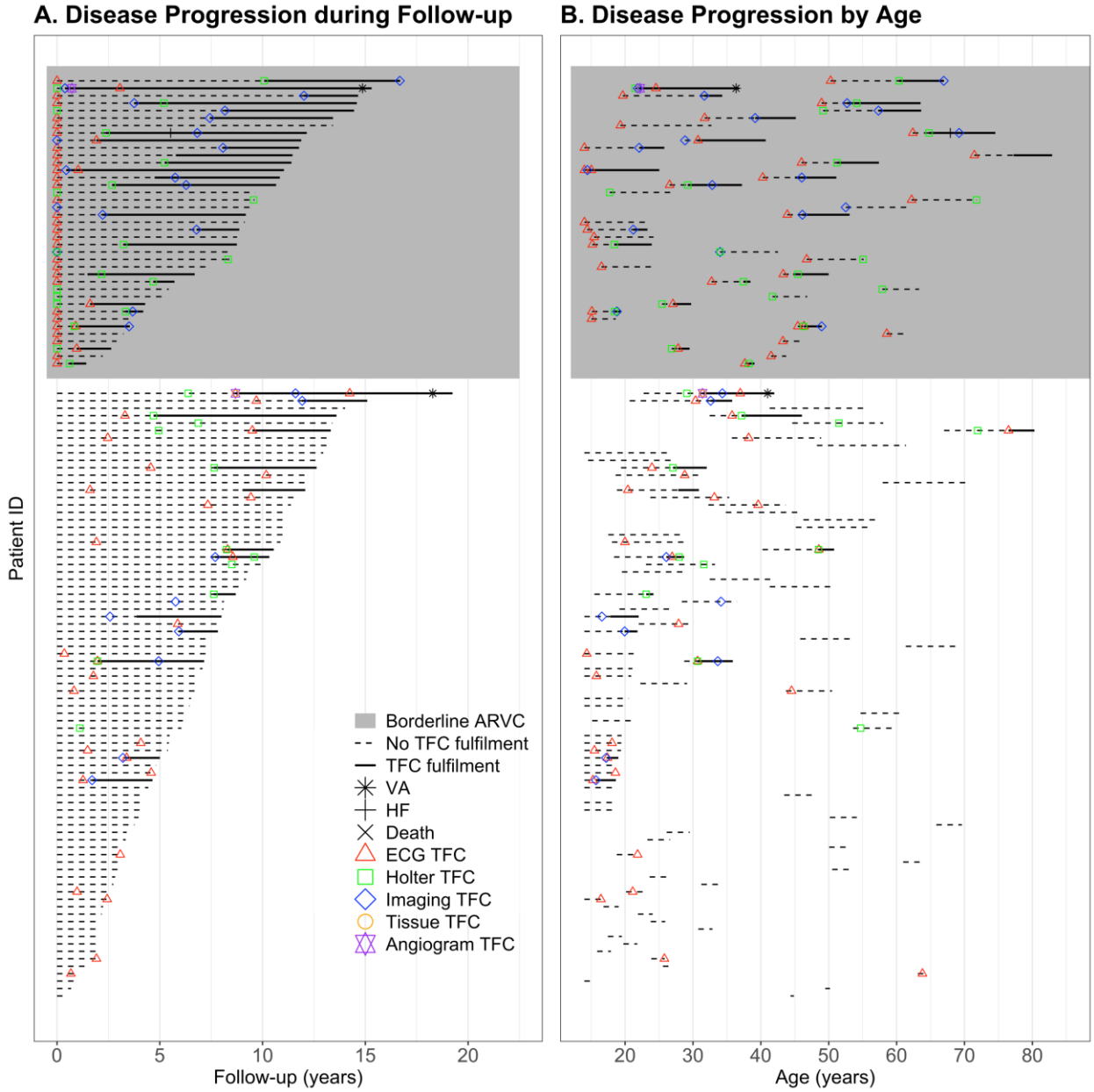
Supplemental Figures

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



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

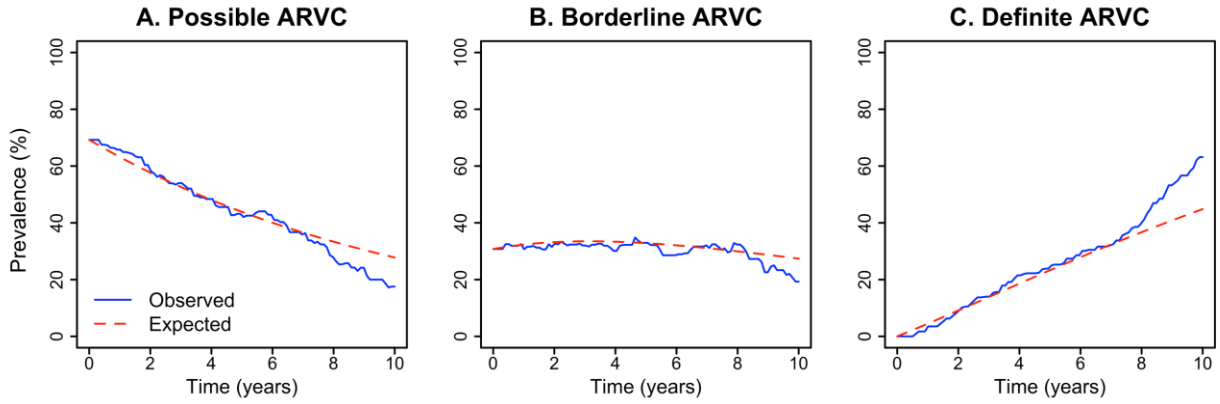
Supplemental Figure 2. Disease progression in the derivation cohort.



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

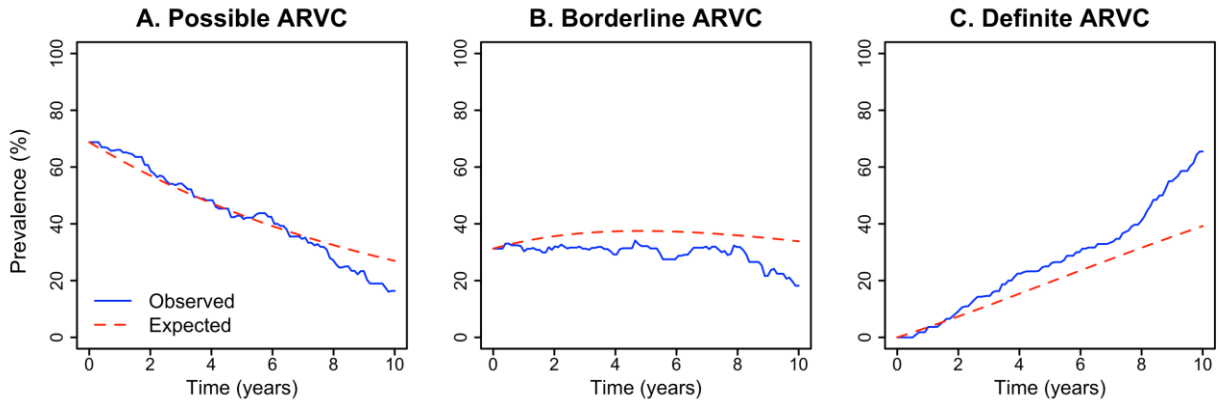
the dashed and solid lines indicates date of diagnosis. A red triangle (ECG), green square (Holter monitor), blue diamond (imaging test), orange circle (tissue) and purple star (angiogram) indicate new TFC of the respective diagnostic test during follow-up. An asterisk, plus sign and multiplication sign 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.



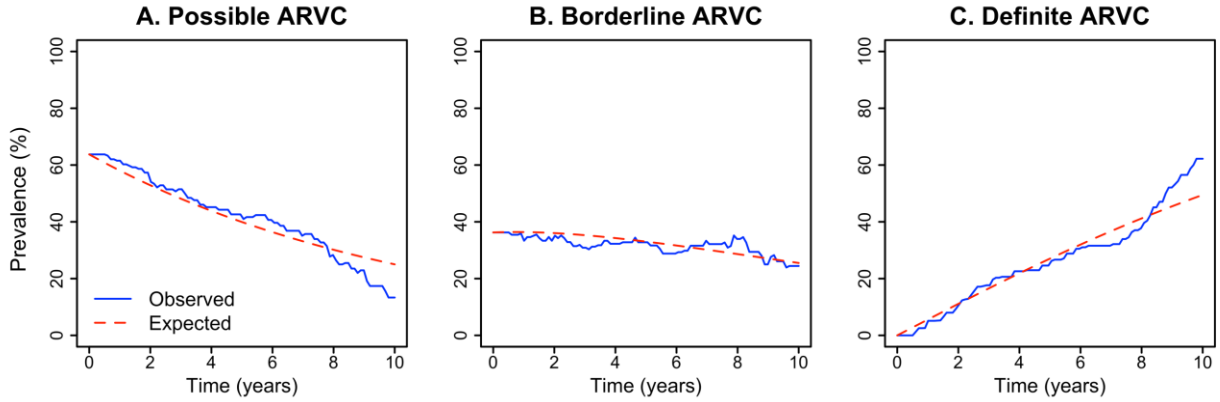
The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse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



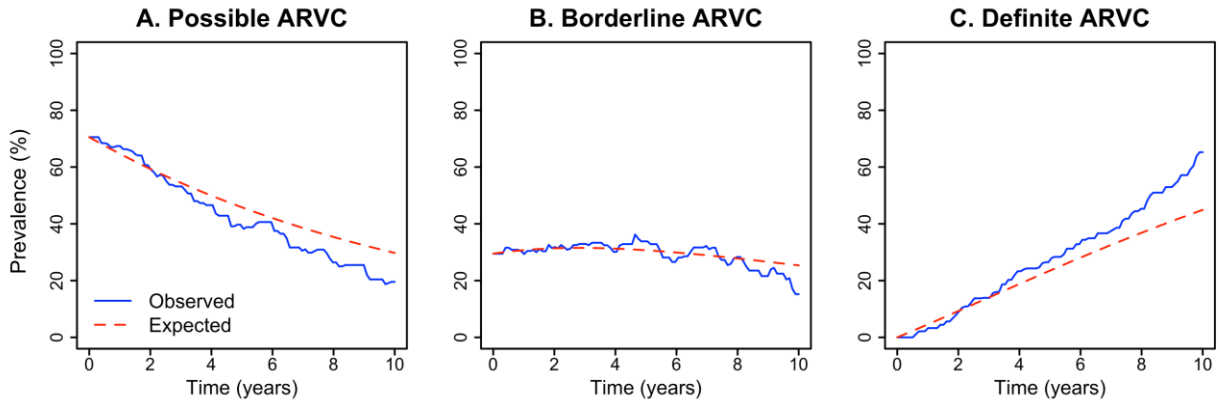
The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse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



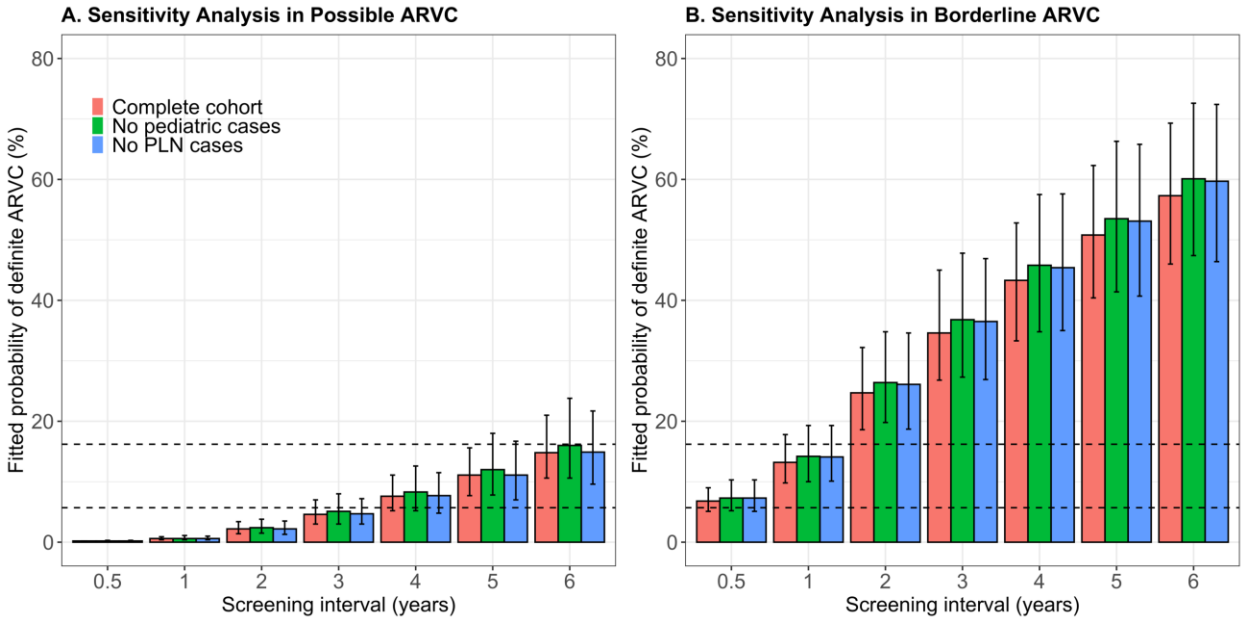
The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse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



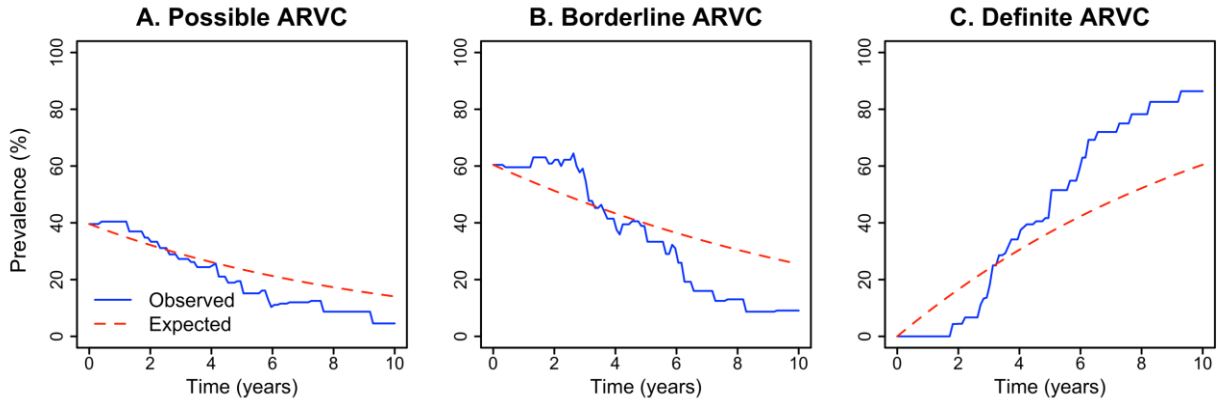
The comparison of the observed (blue line) and expected (red line) is made by a prevalence plot over time in (A) possible ARVC, (B) borderline ARVC, and (C) definite ARVC. The difference between observed and expected progression of disease in the overall study population is not shown as it is the inverse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