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Genetic Variant Score and Arrhythmogenic Right Ventricular Cardiomyopathy Phenotype in Plakophilin-2 Mutation Carriers

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Keywords
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Abstract

Introduction: Whether detailed genetic information contributes to risk stratification of patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) remains uncertain. Pathogenic genetic variants in some genes seem to carry a higher risk for arrhythmia and earlier disease onset than others, but comparisons between variants in the same gene have not been done. Combined Annotation Dependent Depletion (CADD) score is a bioinformatics tool that measures the pathogenicity of each genetic variant. We hypothesized that a higher CADD score is associated with arrhythmic events and earlier age at ARVC manifestations in individuals carrying pathogenic or likely pathogenic genetic variants in plakophilin-2 (*PKP2*). ***Methods:*** CADD scores were calculated using the data from pooled Scandinavian and North American ARVC cohorts, and their association with cardiac events defined as ventricular tachycardia/ventricular fibrillation (VT/VF) or syncope and age at definite ARVC diagnosis were

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assessed. ***Results:*** In total, 33 unique genetic variants were reported in 179 patients (90 males, 71 probands, 96 with definite ARVC diagnosis at a median age of 35 years). Cardiac events were reported in 76 individuals (43%), of whom 53 had sustained VT/VF (35%). The CADD score was neither associated with age at cardiac events (HR 1.002, 95% CI: 0.953–1.054, $p = 0.933$) nor with age at definite ARVC diagnosis (HR 0.992, 95% CI: 0.947–1.039, $p = 0.731$). ***Conclusion:*** No correlation was found between CADD scores and clinical manifestations of ARVC, indicating that the score has no additional risk stratification value among carriers of pathogenic or likely pathogenic *PKP2* genetic variants.

Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heritable heart muscle disease involving primarily the right ventricle (RV). A disease-causing genetic variant is identified in 40–60% of probands [1] and is predominantly related to one of the genes coding for desmosomal proteins, of which plakophilin-2 (*PKP2*) is the most commonly involved [2–5]. ARVC is a progressive disease with large differences in clinical presentation, including ventricular arrhythmias and heart failure, and the diagnosis is based on Task Force Criteria (TFC) proposed in 1994 and modified in 2010 (TFC2010) [6]. Ventricular arrhythmias and sudden cardiac death (SCD) may be the first symptoms [7].

The evidence of early involvement also of the left ventricle in many cases, and sometimes only here, has led to the term "arrhythmogenic cardiomyopathy" (AC or ACM). For this study, we used data from registries evaluating individuals using TFC2010 criteria and have hence kept the term ARVC.

A challenging part of care for patients with ARVC and their families is the counseling regarding the age of disease manifestation in gene variant carriers and the risk of ventricular arrhythmias and SCD [8–11]. Genetic findings may add further knowledge in this area. No convincing evidence of the use of genetic information for risk stratification has been reported for single *PKP2* genetic variants, despite being the most frequent finding. More than 1 pathogenic desmosomal genetic variant in the same individual however seems to be associated with higher arrhythmia risk and earlier progress of the disease [12].

In clinical practice, genetic variants are classified according to the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) guidelines [13]. These guidelines recommend using specific standard terminology for genetic variants in 5 categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. The Combined Annotation Dependent Depletion (CADD) score provides a framework for estimating the relative risk of human genetic variants, and it correlates with both molecular functionality and pathogenicity [14]. The CADD score reflects the difference between the characteristics of genetic variation that is tolerated in the human genome and the characteristics of pathogenic variants, and its predictions are based on a logistic regression model that considers evolutionary conservation, regulatory and transcript information, and protein-level scores. The CADD score integrates 63 different annotations into a single, quantitative score and has been used in the laboratory and previous studies [15, 16] to identify possible pathogenic variants.

However, since the CADD score is not a measure of certainty on whether a genetic variant is pathogenic or not but rather a measure of dysfunctionality on a protein level associated with a specific variant, it appears plausible that this dysfunctionality can be related to the degree of disease manifestations and age at disease penetrance. We have found no study regarding this specific question published so far. The current study intended to investigate, in a cohort of ARVC patients carrying *PKP2* variants, whether the CADD score is associated with clinical manifestations of the disease, including the risk of ventricular arrhythmias.

Methods

The study group is a pooled cohort recruited in 2 prospective observational ARVC registries: the Nordic ARVC Registry and the North American Multidisciplinary Study of ARVC. The Nordic ARVC Register (www.arvc.dk) is an observational register-based study initiated in 2010 that includes patients with definite ARVC by 2010 TFC [6] and their genotype-positive family members enrolled from 9 centers in Denmark, Norway, and Sweden [17]. The North American Multidisciplinary Study of ARVC is also a multicenter study, prospectively gathering information on clinical outcomes, diagnostic measures, and genetics in ARVC probands and their family members [18].

For this study, only individuals carrying a single variant of *PKP2* that was considered pathogenic or likely pathogenic at the time of evaluation were included. The genetic analyses were performed at the discretion of each participating center using the techniques available at the time of evaluation. Even though majority of study participants were genetically assessed recently when NGS technology became available, there are patients who were examined at the

Table 1. Genetic variants in the study, the number of individuals with each score, the number of probands and relatives for each variant, genetic variant type, calculated CADD score for each genetic variant and the cohort(s) in which the variant was found

PKP2 (NM_004572.3) genetic variant	Individuals, *n*	Probands, *n*	Relatives, *n*	Genetic variant type	Cohort	CADD 1.6	ACMG	Classification	gnomAD v2.1.1
c.148_151del ACAG p.(Thr50Serfs*61)	9	6	3	Deletion (fs)	Both	30	PVS1+PS4+PM2+PP5	P	–
c.176A>T p.(Gln59Leu)	1	1	0	Missense	Nordic	24.2	PS4+PP3+BS1	VUS	ALL:0.0085% – NFE:0.0018% – FIN:0.12%
c.218dup p.(Asn74Glnfs*12)	1	1	0	Insertion (fs)	Nordic	29.8	PVS1+PM2+PP3 (+PS4 – 7 individuals)	P	–
c.223+2T>C p.?	1	1	0	Splice site	Nordic	34	PVS1+PM2	LP	–
c.235C>T p.(Arg79*)	14	6	8	Nonsense	Both	38	PVS1+PS4+PM2+PP1+PP5	P	ALL:0.00040% – NFE:0.00090%
c.982_983dupGG p.(Ser329Glufs*24)	5	2	3	Insertion (fs)	Nordic	16.5	PVS1 + PS4 + PM2 + PP1	P	–
c.987delT p.(Ser329Argfs*23)	1	1	0	Deletion	USA	12.4	PVS1+PP5 (PS4 4 individuals)	LP	ALL:0.00040% – AMR:0.0029%
c.1132C>T p.(Gln378*)	2	1	1	Nonsense	USA	42	PVS1+PS4+PM2+PP5	P	ALL:0.00071% – NFE:0.0015%
c.1202_1209delTGCACCTC p.(Leu401Profs*6)	5	3	2	Deletion (fs)	Nordic	33	PVS1+PM2+PP5	P	–
c.1211dup p.(Lys405*)	2	0	2	Insertion	USA	32	PVS1+PS4+PM2+PP5	P	–
c.1219C>T p.(Gln407*)	1	1	0	Nonsense	USA	36	PVS1+PM2	LP	–
c.1355T>A p.(Leu452Ser)	1	1	0	Nonsense	Nordic	39	PVS1+PM2 (PP5 hgmd)	LP	–
c.1378+1G>C p.?	2	1	1	Splice site	Nordic	34	PVS1+PS4+PM2+PP5	P	ALL:0.00040% – NFE:0.00090%
c.1597dup p.(Ile533Asnfs*8)	1	0	1	Insertion (fs)	Nordic	32	PVS1+PM2	P	–
c.1613G>A p.(Trp358*)	1	1	0	Nonsense	USA	44	PVS1+PS4+PP5	P	ALL:0.0016% – ASJ:0.030% – NFE:0.00090%
c.1643del G p.(Gly548Valfs*15)	3	1	2	Deletion (fs)	Nordic	32	PVS1+PS4+PM2+PP5	P	–
c.1688+1G>A p.?	2	0	2	Splice site	USA	33	PVS1+PM2+PP5	P	ALL:0.00040% – NFE:0.00090%
c.1821dupT p.(Val608Cysfs*6)	2	1	1	Insertion (fs)	USA	33	PVS1+PS4+PM2+PP5	P	ALL:0.00040% – NFE:0.00090%
c.1901delA p.(Asn634Thrfs*22)	1	1	0	Deletion (fs)	USA	31	PVS1+PM2+PP5	P	–
c.1952_1955dup p.(Ser652Lysfs*92)	2	1	1	Insertion (fs)	Nordic	33	PVS1+PP5+PM2	P	–
c.1978C>T p.(Gln660*)	2	1	1	Nonsense	USA	40	PVS1+PP5 (PS4 4 individuals)	LP	ALL:0.00071% – EAS:0.010%
c.2013delC p.(Lys672Argfs*12)	2	0	2	Deletion (fs)	USA	28.5	PVS1+PS4+PM2	P	ALL:0.00080% – NFE:0.0018%
c.2057A>G p.(Tyr686Cys)	1	1	0	Missense	USA	26.8	PP3	VUS	ALL:0.00040% – EAS:0.0054%
c.2074A>T p.(Lys692*)	1	1	0	Nonsense	Nordic	38	PVS1+PM2	LP	ALL:0.00040% – NFE:0.00090%
c.2146–1 G>C p.?	66	21	45	Splice site	Nordic	33	PVS1+PS3+PS4+PP1+PP5	P	ALL:0.0032% – NFE:0.0070%
c.2197_2202delCACACCinsG p.(His733Alafs*8)	23	7	16	Insertion (fs)	Nordic	33	PVS1+PS4+PM2+PP5	P	–
c.2197_2201delCACAC p.(His733Profs*8)	5	3	2	Deletion (fs)	USA	32	PVS1+PP5 (PS4 5 individuals)	LP	ALL:0.0021% – NFE:0.0039% – FIN:0.0040%
c.2216_2217delAT p.(His739Argfs*3)	1	1		Deletion (fs)	USA	32	PVS1+PS4+PM2	P	–
c.2300-1G>A p.?	3	1	2	Splice site	Nordic	35	PVS1+PM2	LP	–
c.2386T>C p.(Cys796Arg)	10	1	9	Missense	Nordic	27.2	PS3+PS4+PM2+PP3+PP5	P	–
c.2489+1G>A p.?	2	1	1	Splice site	Nordic	33	PVS1+PS3+PS4+PP1+PP5	P	ALL:0.0028% – AFR:0.0080% – EAS:0.0050% – NFE:0.0039%
c.2509delA p.(Ser837*)	5	2	3	Deletion (fs)	USA	34	PVS1+PS4+PM2+PP5	P	–
c.368G>A p.(Trp123*)	1	1	0	Nonsense	Nordic	36	PVS1+PM2+PP1+PP5 (+PS4 7 individuals)	P	–

ACMG classification and gnomAD values. fs, frame shift; CADD, Combined Annotation Dependent Depletion. Classification: P, pathogenic; LP, likely pathogenic; VUS, variant of unknown significance.

Table 2. Demographic and clinical characteristics of *PKP2* carriers at time of last evaluation (*p* values comparing participants from Nordic ARVC registry to participants from the North American Multidisciplinary ARVC Study)

	All	Nordic	USA	*p* value
N	179	144	35	
CADD, median (IQR)	33.0 (33.0–33.0)	33.0 (33.0–33.0)	33.0 (32.0–38.0)	*0.175*
Male, *n* (%)	90 (50)	71 (49)	19 (54)	*0.598*
Probands, *n* (%)	71 (40)	52 (36)	19 (54)	*0.049*†
Age at ARVC diagnosis, median (IQR)	35 (24–51)	35 (26–53)	31 (22–49)	*0.417*
Age at enrollment, median (IQR)	37 (24–51)	37 (24–52)	37 (24–50)	*0.748*
Age at first cardiac event,* median (IQR)	31 (23–45)	32 (24–44)	26 (20–47)	*0.237*
Age at first sustained VT/VF, median (IQR)	35 (25–46)	36 (25–46)	31 (26–47)	*0.986*
ICD carrier, *n* (%)	82 (46)	59 (41)	23 (66)	*0.012*†
Treatment with beta blocker, *n* (%)	66 (37)	46 (32)	20 (57)	*0.006*†
Treatment with antiarrhythmic drug(s), *n* (%)	50 (28)	39 (27)	11 (31)	*0.608*
VT ablation performed, *n* (%)	19 (11)	14 (10)	5 (14)	*0.539*
Definite ARVC diagnosis according to TFC 2010, *n* (%)	96 (54)	73 (51)	23 (66)	*0.111*
Heart transplantation, *n* (%)	7 (4)	7 (5)	0 (0)	*0.348*
Study endpoints, *n* (%)				
Cardiac events	76 (43)	57 (40)	19 (54)	*0.130*
Sustained VT/VF	64 (36)	52 (37)	11 (31)	*0.563*
Aborted sudden death	12 (7)	10 (7)	2 (6)	*1.000*
Syncope	18 (10)	12 (8)	6 (17)	*0.126*
Years of follow-up, median (IQR)	5.6 (3.3–8.0)	6.3 (4.0–9.0)	3.1 (2.1–4.1)	*0.000*†

Tested with the Mann-Whitney U test (clinical characteristics and outcomes) or Fisher's exact test (frequency of events), significant (†) if $p < 0.05$. ARVC, arrhythmogenic right ventricular cardiomyopathy; CADD, Combined Annotation Dependent Depletion; TFC, Task Force Criteria; SCD, sudden cardiac death; VT, ventricular tachycardia; VF, ventricular fibrillation; ATP, anti-tachycardia pacing; IQR, interquartile range. * Syncope, appropriate ICD therapy (ATP or shock), aborted SCD.

time when the panels were limited to desmosomal genes sets focused on *PKP2*, *DSG2*, *DSC2*, *DSP*, and *JUP* as a standard. The genetic variants reported were for this work classified using ACMG guidelines (American College of Medical Genetics and Genomics) [13]. Those patients who were genotyped before the ACMG guidelines were introduced were re-classified accordingly using the description of the reported genetic variant (Table 1).

The CADD score was calculated using the online calculation provided by the University of Washington and HudsonAlpha Institute for Biotechnology, available at http://cadd.gs.washington.edu/ (version GrCh 37-v1.6). The CADD score calculated for each genetic variant was analyzed for association with study endpoints. For this analysis, we used PHRED-scaled CADD scores as this approach is recommended by the algorithm developers [14].

Baseline clinical and demographic characteristics as well as diagnostic information required by the updated TFC from 2010 [6] were collected. The 2010 Task Force diagnostic criteria were assessed at the time of diagnosis in those who fulfilled the definite ARVC diagnosis requirements or at the last available follow-up in genotype-positive individuals who did not reach the "definite" diagnostic category. Clinical endpoints of interest were (1) sustained ventricular tachycardia (VT) or ventricular fibrillation (VF) defined as either ECG-documented ventricular arrhythmia, appropriate ICD therapy (anti-tachycardia pacing or shock), SCD, or aborted SCD; (2) the first cardiac event defined as syncope or any VT/VF; and (3) the age at definite ARVC diagnosis by the TFC. Patients were censored at the time of the last follow-up.

The Mann-Whitney test was used to compare clinical characteristics and outcomes depending on the CADD score, while Fisher's exact test was used to compare the frequency of events. Receiver operating characteristic curve analysis was performed to assess the relationship between the CADD score and cardiac events, VT/VF, and fulfillment of diagnostic criteria. Data are presented as median and interquartile range (IQR), unless stated otherwise. A p value < 0.05 is considered significant. Statistical analysis was performed with SPSS version 25.0 (IBM SPSS Statistics for Windows, version 25.0. Armonk, NY, USA: IBM Corp).

Results

A total of 179 individuals were identified in the registries as carriers of a pathogenic or likely pathogenic genetic variant in the *PKP2* gene and were included in the study, none of them carrying any other known pathogenic genetic variant in other ARVC-related genes. Table 2 presents clinical characteristics of the study cohort. Vast majority of patients were recruited in Scandinavia ($n = 144$), of whom 99 carried one of the 3 most common genetic variants (Table 1).

Table 3. 2010 Task Force diagnostic criteria in the cohort, prevalence in all individuals and comparison between the 2 participating registries

	All, *n* = 179	Nordic, *n* = 144	USA, *n* = 35	*p* value
Imaging, *n* (%)				
Major	64 (36)	49(34)	15 (43)	*0.330*
Minor	9 (5)	7 (5)	2 (6)	*0.836*
Tissue, *n* (%)				
Biopsy performed	30 (17)	18(13)	12 (34)	
Major*	4 (13)	1 (6)	3 (25)	*0.131*
Minor*	1 (3)	0 (0)	1 (8)	*0.221*
Repolarization, *n* (%)				
Major	60 (34)	47 (33)	13 (37)	*0.614*
Minor	81 (45)	64 (44)	17 (49)	*0.661*
Depolarization, *n* (%)				
Major	7 (4)	6 (4)	1 (3)	*0.721*
Minor	67 (37)	53 (37)	14 (40)	*0.727*
Arrhythmia, *n* (%)				
Major	32 (18)	27 (19)	5 (14)	*0.538*
Minor	68 (38)	53 (37)	15 (43)	*0.509*
Family history, *n* (%)				
Major	179 (100)	144 (100)	35 (100)	*1.000*
Minor	7 (4)	2 (1)	5 (14)	*0.000*†

Tested with the Mann-Whitney U test, significant (†) if $p < 0.05$. * Percentage of those who had biopsy performed.

The CADD score ranged from 12.4 to 44. In the receiver operating characteristic curve analysis, the area under the curve was 0.53 (95% CI 0.44–0.62, $p = 0.526$) for association of the CADD score with cardiac events, 0.50 (95% CI 0.41–0.59, $p = 0.999$) for sustained VT/VF and 0.52 (95% CI 0.43–0.61, $p = 0.661$) for age at ARVC diagnosis. ARVC patients from the North American cohort more often received ICD implant and beta-blocker therapy but otherwise did not show significant differences in phenotypic manifestations of the disease as compared to the Nordic participants (Table 2).

The presence of major and minor TFC 2010 diagnostic criteria is outlined in Table 3. Due to genetic variant carrier status, all individuals in the study fulfilled a major diagnostic criterion from a family history/genetics category.

Table 1 displays the list of unique *PKP2* genetic variants reported in the cohort, the number of individuals carrying each variant and the variants' respective CADD scores. Patients with definite ARVC did not differ in the median CADD score compared to genotype-positive family members who did not reach the definite diagnostic threshold (33.0 [IQR 32.3–33.8] vs. 33.0 [33.0–33.0], $p = 0.406$).

The median CADD score was the same in patients who at any time developed cardiac events as in patients without cardiac events (33.0 [IQR 32.3–33.8] vs. 33.0 [IQR 33.0–33.0], $p = 0.422$, and regarding the sustained VT/VF outcome (33.0 [33.0–33.0] vs. 33.0 [IQR 33.0–33.0], $p = 0.575$). The 33 genetic variants are reported in Table 1. They were splice site mutations ($n = 6$, 18%), nonsense variants ($n = 8$, 24%), deletions ($n = 9$, 27%), missense variants ($n = 3$, 9%), and insertions ($n = 7$, 21%). Seventeen of the variants were only found in the Nordic ARVC registry, 14 were only found in the US cohort, and 2 variants were found in both registries. We did not observe any association between the genetic variant categories in the highest number of individuals (nonsense and deletion) and cardiac events, sustained VT/VF, or age at diagnosis.

Discussion

To the best of our knowledge, this is the first attempt to correlate the CADD score to the ARVC phenotype in *PKP2* mutation carriers. We found no significant correlation between CADD score values and either arrhythmic events or age at ARVC diagnosis.

ARVC patients have an increased risk for ventricular arrhythmic events, including SCD [19, 20]. Several studies of genetic variants intended to use specific genetic information for risk stratification have not proven useful [21, 22], but the number of patients in those studies has often been limited due to relative rarity of the disease.

Reliable estimation of gene variant pathogenicity is a major challenge in clinical genetics, underlined by the introduction of next-generation sequencing in clinical diagnostics. In 2015, the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) proposed guidelines to classify variants in genes associated with Mendelian diseases according to their potential pathogenicity [13]. Reclassification of genetic variants in the ARVC context has been a well-appreciated phenomenon, and the initial interpretation of genetic variant may change with time in both directions. Two of the reported pathogenic genetic variants from our cohort would now be considered benign and were therefore excluded from this study. Two additional variants were at the time of inclusion in the register classified as pathogenic but would now be classified as variant of unknown significance, but not benign, and these are not excluded.

In clinical practice, there is often an issue of whether a given genetic variant is benign or pathogenic. The fairly recent introduced CADD score is described in the reference article from 2014 as a promising alternative or complementary tool [14] and that the method offers a standardized, genome-wide variant scoring metric that incorporates the weighted results of different prediction tools such as PolyPhen and SIFT and the genomic annotation sources such as ENCODE [23]. The resulting CADD score is expressed as a measure of deleteriousness for indels (an insertion or deletion of bases in the genome) and single-nucleotide variants. It is not limited to any specific type of genetic variant or organ systems. The result ranges from 0 to around 45, with no formal upper limit, and no clear cutoff for the point at which a variant is considered pathogenic. A high score represents a variant that is not stabilized by selection and is more often disease-causing than expected at random. By contrast, a low score (single digit) indicates that a variant seems to be an evolutionarily stable, commonly occurring genetic variant with no harm to an organism.

The CADD score has been used as one of several tools to sort out pathogenic variants in studies of simulated whole genomes, not as the single parameter assessing finer gradations in pathogenicity [15, 16]. As of now, there are limited data regarding the clinical use of the scoring system [24], and there are no published studies in which the CADD score has been proven clinically useful as part of the risk stratification in heart diseases.

Genetic variants in *PKP2* are known to be associated with ARVC [25–27]. Genetic variants in *PKP2* may cause ARVC due to desmosomal instability and degradation [25], but newer studies also suggest that other mechanisms, such as interference with intracellular calcium homeostasis [28], may cause ventricular arrhythmias without myocardial structural changes. In the present study, we analyzed all genetic variants reported as pathogenic or likely pathogenic in *PKP2* in the registries using ACMG-AMP guidelines [13]. One can speculate that a high CADD score is due to a genetic variant resulting in a more severely dysfunctional protein that may predict more arrhythmic events in ARVC, and that was our hypothesis for this study.

A recent review and meta-analysis of risk stratification in ARVC provides an overview of risk factors and their predictive potential [29]. Genotype-positive individuals more commonly present with ventricular arrhythmias at a younger age [30]. We also know that patients with pathogenic genetic variants coding for nondesmosomal proteins such as transmembrane protein 43, phospholamban, and lamin A/C seem to be at higher risk for arrhythmia than patients with desmosomal gene variants [31]. Individuals with pathogenic genetic variants in desmoglein-2 is more prone to heart failure than those with variants in *PKP2* [32].

The algorithm of the CADD score cannot consider the presence of >1 genetic variant. In the present study, 9 patients were excluded (6 from the Nordic cohort and 3 from the US cohort) because of carrying double pathogenic genetic variants, 1 in *PKP2* and the other in another ARVC-related gene.

New studies continue to contribute to aiding in risk stratification [19, 33–35]. Apart from different genetic variants, there are other factors known to predispose patients to arrhythmic events, such as physical activity [36–38].

Since there is however significant variability in phenotypic expression and penetrance among individuals with the exact same gene variant [5, 39–41], genetic scores may be used to compare the risk between families with different genetic variants, but not between individuals in the same family. This points out the importance of re-evaluation of genetic analysis done in the past since the knowledge of genetic variants has improved during the last years.

Genetic profiles of patients recruited in the USA differed from the genetic profiles of patients recruited in the Nordic countries (Table 1). Of 33 different genetic variants found, 2 (6%) were present in both the Nordic and the US cohorts, while 17 (52%) were present only in the Nordic individuals and 14 (42%) only in the US cohort. The differences in the use of ICDs and beta-blockers (both ICDs and beta-blockers were more frequently used

in the US cohort) likely reflect the differences in treatment preferences between the regions, since neither the number of individuals with definite ARVC diagnosis nor the incidence of events differs significantly. We chose to perform the Cox regression analysis of the association between the CADD score and age at diagnosis, cardiac events, and sustained VT/VF separately for each cohort without any significant result for any endpoint.

A large proportion of the Nordic cohort carries the variant c.2146-1 G > C which could possibly represent a founder variant common in the Nordic countries; however, at this point, no convincing evidence for that exists. We performed a sensitivity analysis assessing all study endpoints in the study population after exclusion of carriers of this specific variant, which has not affected the risk estimated: HR_{adj} 0.998 95% CI 0.942–1.057 (for sustained VT/VF), HR_{adj} 1.009 95% CI 0.963–1.057 (for definite ARVC diagnosis), and HR_{adj} 1.002 95% CI 0.953–1.053 (for cardiac events).

Study Limitations

As ARVC diagnosis requires an extensive and dedicated diagnostic workup, it is possible that the time to diagnosis is affected by not only the intrinsic factors of the disease mechanisms but also the timing of initiation of diagnostic screening, which may be affected by multiple factors that we cannot account for. This may be particularly important for a family member who may enter the clinical follow-up program without clinical manifestation of the disease and exclusively due to the results of genetic evaluation triggered by events happening to another member of the family.

Conclusions

To the best of our knowledge, this is the first attempt to correlate the CADD score with the ARVC phenotype in carriers of *PKP2* variants. No correlation was found between CADD scores and clinical manifestations of ARVC, indicating that the score has no additional risk stratification value among carriers of pathogenic or likely pathogenic *PKP2* genetic variants.

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Statement of Ethics

Informed consent was obtained from all participants. The study by the Nordic ARVC Register is performed under approval from the Regional Ethics Committee in Lund, Sweden (2010/568 and 2017/485). The study *Genetics, Mechanisms and Clinical Phenotypes of Arrhythmogenic Cardiomyopathy* is approved by the University of Rochester IRB for Coordinating Center (STUDY00000523). The study was also approved by individual enrolling sites. The study complies with the Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.S., P.P., and C.G. contributed to conception and design, and drafting the work. All authors involved in acquisition, analysis, and interpretation of data; critical revision for intellectual content; and approval of the final manuscript version.

Data Availability Statement

Due to patient confidentiality the full data set cannot be made publicly available.

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