Circulation: Genomic and Precision Medicine

ORIGINAL ARTICLE

Arrhythmogenic Right Ventricular Cardiomyopathy-Associated Desmosomal Variants Are Rarely De Novo

Segregation and Haplotype Analysis of a Multinational Cohort

BACKGROUND: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is associated with pathogenic/likely pathogenic (P/LP) variants in genes encoding the cardiac desmosomal proteins. Origin of these variants, including de novo mutation rate and extent of founder versus recurrent variants has implications for variant adjudication and clinical care, yet this has never been systematically investigated.

METHODS: We identified arrhythmogenic right ventricular cardiomyopathy probands who met 2010 Task Force Criteria and had undergone genotyping that included sequencing of the desmosomal genes (*PKP2*, *DSP*, *DSG2*, *DSC2*, and *JUP*) from 3 arrhythmogenic right ventricular cardiomyopathy registries in America and Europe. We classified the desmosomal variants, defined the contribution of unique versus nonunique (ie, not family-specific) P/LP variants, and identified the frequency and characteristics of de novo variants. Next, we haplotyped nonunique variants to determine how often they likely represent a single mutation event in a common ancestor (implied by shared haplotypes) versus multiple mutation events at the same genetic location.

RESULTS: Of 501 arrhythmogenic right ventricular cardiomyopathy probands, 322 (64.3%) carried 327 desmosomal P/LP variants. Most variants (n=247, 75.6%, in 245 patients) were identified in more than one proband and, therefore, considered nonunique. For 212/327 variants (64.8%) genetic cascade screening was performed extensively enough to identify the parental origin of the P/LP variant. Only 3 variants were de novo, 2 of which were whole gene deletions. For 24 nonunique P/LP *PKP2* variants, haplotyping was conducted in 183 available families. For all 24 variants, multiple seemingly unrelated families sharing identical haplotypes were identified, suggesting that these variants originate from common founders.

CONCLUSIONS: Most desmosomal P/LP variants are inherited, nonunique, and originate from ancient founders. Two of 3 de novo variants were large deletions. These observations inform genetic testing, cascade screening, and variant adjudication.

Freyja H.M. van Lint, MD
Brittney Murray, MS
Crystal Tichnell, MGC
Rob Zwart, BS
Nuria Amat, MS
Ronald H. Lekanne Deprez, PhD
Sven Dittmann, PhD
Birgit Stallmeyer, PhD
Hugh Calkins, MD
Jasper J. van der Smagt, MD
Arthur van den Wijngaard, PhD
Dennis Dooijes, PhD
Paul A. van der Zwaag, MD, PhD
Eric Schulze-Bahr, MD, PhD
Daniel P. Judge, MD
Jan D.H. Jongbloed, PhD
J. Peter van Tintelen, MD, PhD
Cynthia A. James, ScM, PhD

Arrhythmogenic right ventricular cardiomyopathy is a genetic cardiomyopathy, characterized histologically by fibrofatty replacement of the myocardium which causes frequent electrical instability and an increased risk of sudden cardiac death.[1] Inheritance of ARVC is classically considered autosomal dominant with age-related, reduced penetrance. Up to 60% of patients with ARVC have pathogenic or likely pathogenic (P/LP) variants[2] in genes encoding the cardiac desmosome (*PKP2*, *DSP*, *DSC2*, *DSG2*, and *JUP*).[3] Pathogenic variants in extra-desmosomal ARVC-associated genes including *CTNNA3*,[4] *PLN*,[5] *TMEM43*,[6] *SCN5A*,[7] *CDH2*,[8] and *DES*[9] have been reported, but are less prevalent.

Even though ARVC is considered an inherited disease, isolated cases are frequent, even in families segregating an established P/LP variant. This may reflect incomplete and age-dependent penetrance. Studies of well-phenotyped families segregating P/LP desmosomal variants document lifetime penetrance of 30% to 50%.[3,10,11] However, the relatively high prevalence of isolated cases also raises the question of how often ARVC is caused by de novo variants, that is, resulting from a spontaneous germline mutation rather than inherited. In some cardiogenetic disorders, including Marfan syndrome, catecholaminergic polymorphic ventricular tachycardia, and vascular Ehlers-Danlos syndrome, the de novo mutation rate is substantial, accounting for 25%, 47%, and 51% of P/LP variants, respectively.[12–14] Although de novo variants in ARVC occasionally have been reported,[9,15–18] the frequency and characteristics have never been systematically studied.

Furthermore, the majority of ARVC probands with desmosomal P/LP variants have recurring, rather than private, family-specific mutations. However, it is largely unknown whether these nonunique (ie, not family-specific) variants identified in multiple, seemingly unrelated families originate from ancient founders or are the result of independent mutation events.[3,19] Several desmosomal ARVC founder variants have been previously identified, for example in the Dutch, Italian, Chinese, and South African populations,[20–24] but these only account for a minority of cases.

Characterizing the origin of desmosomal variants has important implications for clinical care. Establishing the de novo mutation rate will inform genetic counseling and approaches to cascade screening. Furthermore, the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines for variant interpretation designate both de novo status and identification in multiple unrelated probands with the same phenotype as criteria towards pathogenicity.[2,25] Establishing the origin of desmosomal variants will thus inform evaluation for the pathogenicity of rare desmosomal variants which is particularly important as most individual ARVC families have too few cases for conclusive segregation studies.

Therefore, leveraging a multinational cohort of ARVC probands with P/LP desmosomal variants and their families, we conducted a 2-phase study to characterize the origin of ARVC-associated desmosomal variants. First, we cataloged the variants, defined the contribution of unique versus nonunique (ie, not family-specific) variants, and identified the frequency and characteristics of de novo variants. Next, we haplotyped nonunique variants to determine how often they likely represent a single mutation event in a common ancestor (implied by shared haplotypes) versus multiple mutation events at the same genetic location (implied by the variant occurring on multiple different haplotypes).

METHODS

The data supporting study findings and methods used in analysis are available from the corresponding author on reasonable request. The project was approved by the respective institutional review boards of the involved centers, and participants provided informed consent as per institutional protocol. The full methods are available as material in the Data Supplement.

RESULTS

Study Population

Ascertainment of the study population is shown in Figure 1. The study population consisted of 501 probands with definite ARVC. Of these, 322 (64.3%) carried at least one P/LP desmosomal. In 34 (6.8%) probands, P/LP variants had been identified in nondesmosomal genes including *PLN* (n=26), *SCN5A* (n=5), *TMEM43* (n=1), or a sarcomere gene (n=2). The remaining probands were without a known genetic cause for their ARVC.

The clinical and demographic characteristics of the 322 desmosomal variant carrying probands included in this study are summarized in Table 1. Approximately half (n=171, 53.1%), were from the Johns Hopkins University (JHU) Registry, 122 probands (37.9%) were from the Netherlands Heart Institute (NLHI) Registry, with the remainder (n=29, 9.0%) from the Münster University Hospital (MUH) Registry. Most probands were male (64.9%) and white (97.5%). They presented at a median 34±15 years, typically with an arrhythmic event.

Genotype and Variant Classification

The 2-step process of adjudication of variants resulted in 12 discrepancies between the initial adjudication and re-review. As detailed in Table II in the Data Supplement, all but one of these variants were in *DSC2*, *DSG2*, or *DSP*. Most were loss-of-function variants initially categorized as a variant of uncertain significance and upgraded to LP based on application of the American College of Medical Genetics and Genomics/Association for Molecular Pathology PVS1 criterion (null
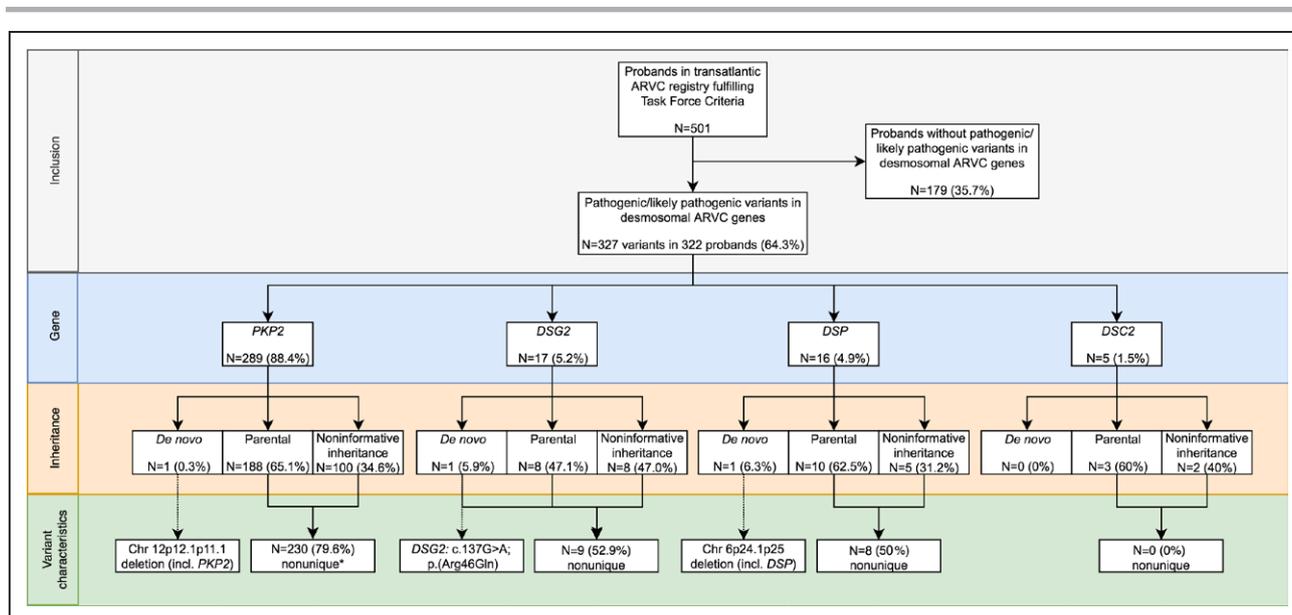
Figure 1. Inheritance of variants.
Tree diagram summarizing the inheritance of desmosomal variants in probands with arrhythmogenic right ventricular cardiomyopathy. ARVC indicates arrhythmogenic right ventricular cardiomyopathy; and Chr, chromosome. *230 probands carried 26 different nonunique variants in *PKP2*. For 24 nonunique variants, haplotype analysis has been performed in 183 families using 260 samples.

variant in gene with established loss-of-function as disease mechanism) and inclusion of segregation data from our registries.

The 322 probands had 327 P/LP desmosomal variants (5 patients carried 2 P/LP desmosomal variants). As shown in Figure 1, most variants (n=289, 88.4%) were found in *PKP2* with substantially fewer in *DSG2* (n=17), *DSP* (n=16), and *DSC2* (n=5). Table II in the Data Supplement lists variants in these patients. Most were splice-site or truncating variants (n=287, 87.5%). Deletions encompassing one or more exons were seen in 13 patients (4.0%), including 3 whole gene deletions (2 *PKP2* and one *DSP*).

Most variants (n=247, 75.5%) were found in more than one proband and, therefore, were considered nonunique. Most nonunique variants were in *PKP2* (n=230/247, 93.1%). Nonunique *PKP2* variants encompassed 26 genotypes. Half of nonunique variants (14/26, 54%) were shared by probands from more than one country (Table II in the Data Supplement).

Inheritance

In 209 probands (64.9%, 212 variants), cascade screening was performed that allowed for determination of inheritance. Extent of cascade testing is shown in Figure I in the Data Supplement.

Only 3 variants were apparently de novo (1.4%): a deletion encompassing *PKP2*, a deletion encompassing *DSP*, and a missense variant c.137G>A; p.(Arg46Gln) in *DSG2*. Thus, de novo variants in our population were disproportionately whole gene deletions (2 de novo variants [66.7%] versus one inherited variant [0.5%],

P<0.001). Both cases harboring a de novo whole gene deletion have been described in detail previously.[16,17] Paternity was confirmed for the probands with the *PKP2* and *DSP* variants. For the first, all highly polymorphic markers were informative, and for the latter, cosegregation of another rare familial variant was considered informative (*PKP2*: c.2197_2202delinsG; p.His733Alafs*). The de novo missense variant, *DSG2* c.137G>A; p.(Arg46Gln), has been previously suggested to be a founder variant,[26] but additional testing to confirm nonpaternity or mosaicism could not be performed due to limited consent for genetic analysis.

In 114 probands (115 variants), it was not possible to determine inheritance. In most cases, inheritance was undeterminable because either no cascade screening had been performed (n=72, 63.2%), or genotyping was only done in younger generations in the pedigree (eg, children, grandchildren, n=27, 23.7%). For the remaining 15 probands (13.1%), familial cascade testing had been performed in family members, but was negative. As shown in Table 2, within this group, only 2 had a family history suggestive for ARVC, and therefore the 13 patients without a suggestive family history (86.7%) could potentially harbor undetected de novo variants. However, compared with the other groups without informative cascade screening, there was no statistically significant difference for this group to meet any of the major or minor family criteria.

Haplotype Analyses

Haplotype analyses were performed for 24 different P/LP nonunique *PKP2* variants in 183 families with ARVC

Table 1. Clinical Characteristics of the Study Population

Clinical Characteristics (N=322)	N (%)
Male	209 (64.9)
Proband	322 (100)
Ethnic background	
White	314 (97.5)
African/African-American	3 (0.9)
Asian	5 (1.6)
Presentation	
Age at presentation, y	34±15
Type of presentation	
Sudden cardiac death	11 (3.4)
Resuscitated	28 (8.7)
Sustained VT	145 (45)
2010 Task Force Criteria met	
Structural alterations	
Major	185/306 (60.5)
Minor	36/306 (11.8)
Repolarization abnormalities	
Major	243/298 (81.5)
Minor	16/298 (5.4)
Depolarization abnormalities	
Major	31/281 (11)
Minor	129/281 (45.9)
Arrhythmias	
Major	121/291 (41.6)
Minor	127/291 (43.6)
Family history/genetics	
Major	322 (100)
Events at last follow-up	
ICD implantation	231/314 (73.6)
Sustained VT/VF	189/319 (59.2)
Cardiac transplantation	10/317 (3.2)
Death	23/318 (7.2)
Follow-up duration [interquartile range]	7 [13]

ICD indicates implantable cardioverter defibrillator; VF, ventricular fibrillation; and VT, ventricular tachycardia;

(66 JHU, 90 NLHI, and 27 MUH) to determine whether these nonunique variants have shared haplotypes.

As shown in Figure 2, for all 24 P/LP nonunique variants, shared haplotypes were identified in multiple seemingly unrelated families. In Figure 2, number of unique haplotypes for each variant are displayed by colored dots. As can be appreciated, for most variants the number of haplotypes is far smaller than the number of families studied. For instance, *PKP2* c.235C>T; p.Arg79* was haplotyped in 30 families, and only 3 unique haplotypes were detected: one shared among the JHU, NLHI, and MUH registries (red dot), and 2

additional (blue and orange dots) in both the NLHI and MUH registries. It is notable that for the second most common *PKP2* variant in this cohort (c.2146-1G>C; p.Met716fs) only 2 haplotypes were identified, with one major haplotype shared across JHU, NLHI. and MUH. Full details of haplotype analyses are provided in Table III in the Data Supplement.

DISCUSSION

Main Findings

This study assessed the origin of ARVC-associated P/LP desmosomal variants and has 2 main findings. First, most P/LP desmosomal variants in ARVC are nonunique (75.3%), that is, occurring in multiple families and inherited (98.6%). Second, most nonunique *PKP2* variants share haplotypes. Taken together these results suggest most ARVC-associated variants originate from common founders.

Prior Studies

Early publications on the genetics of ARVC identified the cardiac desmosome as the central structure underlying ARVC pathogenesis.[28,29] Numerous P/LP variants have now been reported in each desmosomal gene.[19] The presence of identical variants in seemingly unrelated ARVC probands was also noted in early publications.[28] This raised the question of whether these nonunique variants reflected mutation hotspots in desmosomal genes or instead were the result of ancient founder mutations. Already in 2006, Van Tintelen et al[27] described shared haplotypes in carriers of several *PKP2* P/LP variants. Additional desmosomal founder variants have been described in the Dutch, Italian, Chinese, and South African ARVC populations.[20–24] Founder mutations have also been extensively studied in hypertrophic cardiomyopathy and long QT syndrome.[30,31] A recent analysis of nonunique variants in an Australian hypertrophic cardiomyopathy population showed that in contrast to the results presented here, nonunique *MYH7* and *MYBPC3* variants were likely the result of recurrent mutation events.[32]

In contrast to founder mutations, mutations may also occur de novo. De novo mutations may recur, resulting in unrelated families with identical P/LP variants on different haplotypes. Alternately, de novo mutation events may occur in a novel location resulting in a unique variant. For some cardiovascular disorders, such as Marfan syndrome, vascular Ehlers-Danlos syndrome, and catecholaminergic polymorphic ventricular tachycardia, de novo mutations account for up to half of variants identified.[12–14] In Marfan syndrome, these de novo mutations tend to be unique, however in other genetic disorders, (eg, achondroplasia) recurrent de novo muta-

Table 2. Family History per 2010 Task Force Criteria in Families With Noninformative Inheritance Stratified by Extent of Cascade Genetic Testing

Extent of cascade testing	N (%)	Major Family History Criteria		Minor Family History Criteria	
		ARVC confirmed in a first-degree relative who meets TFC	ARVC confirmed pathologically at autopsy or surgery in a first-degree relative	Premature sudden death<age 35 due to suspected ARVC in a first-degree relative	History of ARVC in a first-degree relative in whom it is not possible to determine whether the family member meets TFC 2010
None	72 (63.2)	1 (1.4)	1 (1.4)	6 (8.3)	3 (4.2)
Only lower generations	27 (23.7)	1 (3.7)	0 (0)	2 (7.4)	3 (11.1)
≥1 potentially informative relative tested negative*	15 (13.1)	0 (0)	0 (0)	2 (13.3)	0 (0)
Total	114 (100)	2 (1.8)	1 (0.9)	10 (8.8)	6 (5.3)

ARVC indicates arrhythmogenic right ventricular cardiomyopathy; and TFC, task force criteria.

*No statistically significant differences were observed in the likelihood of meeting any of the major or minor family history criteria between families in which one or more potentially informative relative tested negative and families without any cascade testing or only in the lower generations to (P=1.000, P=1.000, P=0.618, and P=1.000).

tions at hotspots are the rule.[33] Several unique de novo variants in ARVC have been previously described.[9,15–17]

The results presented here build on this literature by establishing the genetic origin of desmosomal P/LP variants in the largest population of ARVC probands to date. In doing so, we establish that de novo variants in ARVC are rare. When they occur, de novo variants seem to be disproportionately part of large deletions rather than recurrent mutations. In contrast, nonunique variants with shared haplotypes suggesting founder variants are common. The relatively few founder variants previously described in the literature is likely a substantial underestimate of their actual contribution to the pathogenesis of ARVC.

Figure 2. Haplotyped variants.
Colors indicate haplotypes per variant, that is, multiple colors refer to multiple haplotypes. dbSNP indicates reference number in the Single Nucleotide Polymorphism Database; FM, family member; JHU, Johns Hopkins University; MUH, Münster University Hospital; NLHI, Netherlands Heart Institute; and P, proband. Variants for which haplotype analyses have been performed previously: c.235C>T; p.(Arg79*)[21], c.397C>T; p.(Gln133*)[27], c.1132C>T; p.(Gln378*)[20], c.2146-1G>C; p.(Met716fs)[28], c.2386T>C; p.(Cys796ArG)[27] and c.2489+1G>A; p.?[27].

Clinical Implications

The results of this study have important implications for clinical care. The high percentage of inherited variants will inform genetic counseling and approaches to cascade screening. Specifically, anticipatory guidance given during pretest counseling should include the likelihood that a variant, if identified, has very likely been inherited. Thus, signs or symptoms in a relative should be considered with a high index of suspicion.

In rare cases where a de novo variant is identified, parents and siblings are highly unlikely to be at risk. However, one has to keep in mind that this risk is not 0%, as mosaicism has been described previously in cardiogenetic diseases.[34] While not the primary goal of this analysis, it is also worth emphasizing that 13 (4.0%) of the P/LP variants in this study were large deletions. While these large deletions in ARVC have been previously reported[16,17,35] our findings highlight the importance of using a genetic test capable of identifying deletions.

These data also provide perspective on classification of desmosomal variants using the American College of Medical Genetics and Genomics/Association for Molecular Pathology criteria. Variant classification remains a challenge in ARVC due to the relative rarity of the condition combined with the large number of reported desmosomal variants. The American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines for variant interpretation designate de novo status as a strong indication for pathogenicity. Our data show this criterion will unfortunately have limited utility in adjudication of desmosomal variants, since only a small percentage is de novo. On the contrary, the identification of an identical variant in multiple unrelated probands (proband count), recently proposed for variant interpretation in *MYH7* related cardiomyopathies,[2] has promise for contributing to the classification of desmosomal variants.

Implications for Population Penetrance

Our findings also have interesting implications for understanding population-based penetrance of desmosomal variants. Among the nonunique variants in *PKP2* in this study, most are on shared haplotypes, suggesting they originate from common ancient founders. This observation is particularly notable as haplotypes were shared among probands from different continents. It indicates these variants were maintained in the population throughout history. This is surprising given ARVC has an elevated risk of sudden cardiac death beginning at puberty and an average age of sudden cardiac death presentation in the mid to late 20s among desmosomal variant carriers.[36,37] This being the reproductive age, one could anticipate that reproductive fitness is impaired. However, if reproductive fitness was substantially impaired, selection against the variant and subsequent gradual elimination from the population would be expected. Maintenance of the variant in the population could reflect a much lower population penetrance than what we know from well-phenotyped ARVC families in which desmosomal variant carriers have a life time penetrance of 30% to 50%. Evidence is emerging that this lower population penetrance may be the case.[38] A recent study of P/LP desmosomal variant carriers ascertained from the general population suggested extremely low penetrance, estimated at 5%.[39] Furthermore, growing evidence suggests ARVC penetrance may require multiple hits—both genetic and environmental—to reach a threshold for disease expression. Vigorous endurance exercise increases penetrance in ARVC families.[40] Understanding population penetrance of P/LP desmosomal variants is an increasingly important issue in the era of return of ARVC-associated variants as secondary findings.[41]

Limitations

Only 64.9% of probands had undergone genetic cascade screening in their family extensively enough to identify the origin of the P/LP variant, as indicated in Figure 1. This likely reflects challenges of cascade screening encountered in daily practice.[42] However, families without informative cascade genetic testing could harbor unidentified de novo variants. Next, inheritance and contribution of different genes associated with ARVC may differ based on ethnicity. While this study included probands from 2 European countries and the more multi-ethnic North American population, the majority of probands had European ancestry. This may reflect a true higher prevalence of ARVC-associated desmosomal variants in this population or may reflect ascertainment bias in the Registries. In addition, sharing of haplotypes in Europe and America is not surprising as, since the 1820s, more than 40 million European citizens emigrated to America.[43] This includes over 400 000 immigrants with their last known residence in the Netherlands and >7 million immigrants from Germany. A cohort with a larger group of individuals with other ethnic backgrounds could be expected to have less haplotype sharing.

CONCLUSIONS

Most desmosomal P/LP variants are inherited, nonunique, and often originate from ancient founders. Two out of 3 de novo variants were deletions encompassing an entire gene. These observations inform genetic cascade screening and variant classification in ARVC. Furthermore, they set the stage for understanding true penetrance of disease in the era of widespread availability of genetic testing.

REFERENCES

1. Gandjbakhch E, et al. Clinical diagnosis, imaging, and genetics of arrhythmogenic right ventricular cardiomyopathy/dysplasia: JACC State-of-the-art review. *J Am Coll Cardiol*. 2018;72:784–804.
2. Richards S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. doi: 10.1038/gim.2015.30
3. Groeneweg JA, et al. Clinical presentation, long-term follow-up, and outcomes of 1001 arrhythmogenic right ventricular dysplasia/cardiomyopathy patients and family members. *Circ Cardiovasc Genet*. 2015;8:437–446. doi: 10.1161/CIRCGENETICS.114.001003
4. van Hengel J, et al. Mutations in the area composita protein αT-catenin are associated with arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J*. 2013;34:201–210. doi: 10.1093/eurheartj/ehs373
5. van der Zwaag PA, et al. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. *Eur J Heart Fail*. 2012;14:1199–207.
6. Haywood AF, et al. Recurrent missense mutations in TMEM43 (ARVD5) due to founder effects cause arrhythmogenic cardiomyopathies in the UK and Canada. *Eur Heart J*. 2013;34:1002–1011.
7. Te Riele AS, et al. Multilevel analyses of SCN5A mutations in arrhythmogenic right ventricular dysplasia/cardiomyopathy suggest non-canonical mechanisms for disease pathogenesis. *Cardiovasc Res*. 2017;113:102–11.
8. Mayosi BM, et al. Identification of cadherin 2 (CDH2) mutations in arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet*. 2017;10:e001605.
9. Klauke B, et al. De novo desmin-mutation N116S is associated with arrhythmogenic right ventricular cardiomyopathy. *Hum Mol Genet*. 2010;19:4595–4607. doi: 10.1093/hmg/ddq387
10. Protonotarios N, et al. Arrhythmogenic right ventricular cardiomyopathy/dysplasia on the basis of the revised diagnostic criteria in affected families with desmosomal mutations. *Eur Heart J*. 2011;32:1097–1104. doi: 10.1093/eurheartj/ehr043
11. Quarta G, et al. Familial evaluation in arrhythmogenic right ventricular cardiomyopathy: impact of genetics and revised task force criteria. *Circulation*. 2011;123:2701–2709. doi: 10.1161/CIRCULATIONAHA.110.976936
12. Judge DP, et al. Marfan's syndrome. *Lancet*. 2005;366:1965–1976. doi: 10.1016/S0140-6736(05)67789-6
13. Ohno S, et al. Gender differences in the inheritance mode of RYR2 mutations in catecholaminergic polymorphic ventricular tachycardia patients. *PLoS One*. 2015;10:e0131517. doi: 10.1371/journal.pone.0131517
14. Legrand A, et al. Frequency of de novo variants and parental mosaicism in vascular Ehlers-Danlos syndrome. *Genet Med*. 2019;21:1568–1575. doi: 10.1038/s41436-018-0356-2
15. Gandjbakhch E, et al. Sporadic arrhythmogenic right ventricular cardiomyopathy/dysplasia due to a de novo mutation. *Europace*. 2009;11:379–381. doi: 10.1093/europace/eun378
16. Murray B, et al. A family with a complex clinical presentation characterized by arrhythmogenic right ventricular dysplasia/cardiomyopathy and features of branchio-oculo-facial syndrome. *Am J Med Genet A*. 2013;161A:371–376. doi: 10.1002/ajmg.a.35733
17. Roberts JD, et al. Detection of genomic deletions of PKP2 in arrhythmogenic right ventricular cardiomyopathy. *Clin Genet*. 2013;83:452–456. doi: 10.1111/j.1399-0004.2012.01950.x
18. Ingles J, et al. Concealed arrhythmogenic right ventricular cardiomyopathy in sudden unexplained cardiac death events. *Circ Genom Precis Med*. 2018;11:e002355. doi: 10.1161/CIRCGEN.118.002355
19. Lazzarini E, et al. The ARVD/C genetic variants database: 2014 update. *Hum Mutat*. 2015;36:403–410. doi: 10.1002/humu.22765
20. Watkins DA, et al; ARVC Registry of the Cardiac Arrhythmia Society of Southern Africa (CASSA). Clinical features, survival experience, and profile of plakophylin-2 gene mutations in participants of the arrhythmogenic right ventricular cardiomyopathy registry of South Africa. *Heart Rhythm*. 2009;6(11 suppl):S10–S17. doi: 10.1016/j.hrthm.2009.08.018
21. van der Zwaag PA, et al. Recurrent and founder mutations in the Netherlands: Plakophilin-2 p.Arg79X mutation causing arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Neth Heart J*. 2010;18:583–591. doi: 10.1007/s12471-010-0839-5
22. van der Smagt JJ, et al. Clinical and genetic characterization of patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy caused by a plakophilin-2 splice mutation. *Cardiology*. 2012;123:181–189. doi: 10.1159/000342717
23. Lorenzon A, et al. Homozygous desmocollin-2 mutations and arrhythmogenic cardiomyopathy. *Am J Cardiol*. 2015;116:1245–1251. doi: 10.1016/j.amjcard.2015.07.037
24. Chen L, et al. A founder homozygous DSG2 variant in East Asia results in ARVC with full penetrance and heart failure phenotype. *Int J Cardiol*. 2019;274:263–270. doi: 10.1016/j.ijcard.2018.06.105
25. Kelly MA, et al. Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies: recommendations by ClinGen's Inherited Cardiomyopathy Expert Panel. *Genet Med*. 2018;20:351–359. doi: 10.1038/gim.2017.218
26. Bhuiyan ZA, et al. Desmoglein-2 and desmocollin-2 mutations in dutch arrhythmogenic right ventricular dysplasia/cardiomypathy patients: results

from a multicenter study. *Circ Cardiovasc Genet.* 2009;2:418–427. doi: 10.1161/CIRCGENETICS.108.839829

27. van Tintelen JP, et al. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation.* 2006;113:1650–1658. doi: 10.1161/CIRCULATIONAHA.105.609719

28. Gerull B, et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet.* 2004;36:1162–1164. doi: 10.1038/ng1461

29. Protonotarios N, et al. Genotype-phenotype assessment in autosomal recessive arrhythmogenic right ventricular cardiomyopathy (Naxos disease) caused by a deletion in plakoglobin. *J Am Coll Cardiol.* 2001;38:1477–1484. doi: 10.1016/s0735-1097(01)01568-6

30. Page SP, et al. Cardiac myosin binding protein-C mutations in families with hypertrophic cardiomyopathy: disease expression in relation to age, gender, and long term outcome. *Circ Cardiovasc Genet.* 2012;5:156–166. doi: 10.1161/CIRCGENETICS.111.960831

31. Christiansen M, et al. Mutations in Danish patients with long QT syndrome and the identification of a large founder family with p.F29L in KCNH2. *BMC Med Genet.* 2014;15:31. doi: 10.1186/1471-2350-15-31

32. Ross SB, et al. Burden of recurrent and ancestral mutations in families with hypertrophic cardiomyopathy. *Circ Cardiovasc Genet.* 2017;10:e001671.

33. Gomes MES, et al. Novel and recurrent mutations in the FGFR3 gene and double heterozygosity cases in a cohort of brazilian patients with skeletal dysplasia. *Mol Syndromol.* 2018;9:92–99. doi: 10.1159/000486697

34. Forissier JF, et al. First description of germline mosaicism in familial hypertrophic cardiomyopathy. *J Med Genet.* 2000;37:132–134. doi: 10.1136/jmg.37.2.132

35. Pilichou K, et al. Large genomic rearrangements of desmosomal genes in italian arrhythmogenic cardiomyopathy patients. *Circ Arrhythm Electrophysiol.* 2017;10:e005324.

36. Bhonsale A, et al. Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. *Eur Heart J.* 2015;36:847–855. doi: 10.1093/eurheartj/ehu509

37. Gupta R, et al. Comparison of features of fatal versus nonfatal cardiac arrest in patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Am J Cardiol.* 2017;120:111–117. doi: 10.1016/j.amjcard.2017.03.251

38. Haggerty CM, et al. Electronic health record phenotype in subjects with genetic variants associated with arrhythmogenic right ventricular cardiomyopathy: a study of 30,716 subjects with exome sequencing. *Genet Med.* 2017;19:1245–1252. doi: 10.1038/gim.2017.40

39. Haggerty CM, et al. Prevalence and electronic health record-based phenotype of loss-of-function genetic variants in arrhythmogenic cardiomyopathy-associated genes in 61,000 individuals. *Circulation.* 2018;138:A15371.

40. James CA, 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;62:1290–1297. doi: 10.1016/j.jacc.2013.06.033

41. Haggerty CM, et al. Managing secondary genomic findings associated with arrhythmogenic right ventricular cardiomyopathy: case studies and proposal for clinical surveillance. *Circ Genom Precis Med.* 2018;11:e002237. doi: 10.1161/CIRCGEN.118.002237

42. Burns C, James C, Ingles J. Communication of genetic information to families with inherited rhythm disorders. *Heart Rhythm.* 2018;15:780–786. doi: 10.1016/j.hrthm.2017.11.024

43. Department of Homeland Security, United States. *Yearbook of Immigration Statistics: 2013*. U.S. Department of Homeland Security, Office of Immigration Statistics: Washington, DC;2014.