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ORIGINAL ARTICLE

Cadherin 2-Related Arrhythmogenic Cardiomyopathy Prevalence and Clinical Features

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This manuscript is dedicated to the memory of Professor Bongani M. Mayosi (28 January 1967 – 27 July 2018), Professor of Cardiology and Dean of the Faculty of Health Sciences at the University of Cape Town, South Africa. This study was inspired by him and made possible because of his relentless and successful search for the genetic cause of ACM in a family that he had personally followed for 20 years. He is sorely missed.

BACKGROUND: Arrhythmogenic cardiomyopathy (ACM) is an inherited cardiac disease characterized by fibrofatty replacement of the right and left ventricle, often causing ventricular dysfunction and life-threatening arrhythmias. Variants in desmosomal genes account for up to 60% of cases. Our objective was to establish the prevalence and clinical features of ACM stemming from pathogenic variants in the nondesmosomal cadherin 2 (CDH2), a novel genetic substrate of ACM.

METHODS: A cohort of 500 unrelated patients with a definite diagnosis of ACM and no disease-causing variants in the main ACM genes was assembled. Genetic screening of CDH2 was performed through next-generation or Sanger sequencing. Whenever possible, cascade screening was initiated in the families of CDH2-positive probands, and clinical evaluation was performed.

RESULTS: Genetic screening of CDH2 led to the identification of 7 rare variants: 5, identified in 6 probands, were classified as pathogenic or likely pathogenic. The previously established p.D407N pathogenic variant was detected in 2 additional probands. Probands and family members with pathogenic/likely pathogenic variants in CDH2 were clinically evaluated, and along with previously published cases, altogether contributed to the identification of gene-specific features (13 cases from this cohort and 11 previously published, for a total of 9 probands and 15 family members). Ventricular arrhythmic events occurred in most CDH2-positive subjects (20/24, 83%), while the occurrence of heart failure was rare (2/24, 8.3%). Among probands, sustained ventricular tachycardia and sudden cardiac death occurred in 5/9 (56%).

CONCLUSIONS: In this worldwide cohort of previously genotype-negative ACM patients, the prevalence of probands with CDH2 pathogenic/likely pathogenic variants was 1.2% (6/500). Our data show that this cohort of CDH2-ACM patients has a high incidence of ventricular arrhythmias, while evolution toward heart failure is rare.

Key Words: cadherins ■ cardiomyopathy ■ mutation ■ sudden cardiac death ■ tachycardia

Arrhythmogenic right ventricular (RV) cardiomyopathy is an inherited cardiac disease characterized by fibrofatty replacement of the RV, accompanied by electrical instability predisposing to ventricular arrhythmias and sudden cardiac death.^{1,2} The major form of the disease (arrhythmogenic RV

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Nonstandard Abbreviations and Acronyms

ACM	arrhythmogenic cardiomyopathy
EC	ectodomain
EP	electrophysiological study
PVC	premature ventricular complexes
RV	right ventricle
VT	ventricular tachycardia

cardiomyopathy) is characterized by a predominant RV involvement. However, given the evidence of forms with biventricular or predominant left ventricular involvement,³ the term arrhythmogenic cardiomyopathy (ACM) is now preferred.^{4,5}

ACM is mainly caused by variants in genes encoding proteins of the desmosome (*PKP2*, *DSP*, *DSG2*, *DSC2*, and *JUP*), a specialized junction involved in cell adhesion.^{5,6} Desmosomes, together with the fascia adherens junctions and the gap junctions, are located at the intercalated discs, which maintain a tight connection between cardiomyocytes and allow cellular communication. In mammals, these 3 functional structures are found in close proximity⁷ and form a hybrid zone that operates as a single functional network known as the area composita.^{8,9} Nowadays, the idea of ACM being a disease whose functional basis extends beyond the desmosome to the wider zone of the intercalated discs is gaining interest.^{10,11} In a previous study by Mayosi et al,¹² we identified clinically actionable variants in the *CDH2* gene, encoding cadherin 2, a major structural component of fascia adherens junctions, as a novel genetic substrate of ACM.¹³ The concept that the genetic basis of ACM extends to nondesmosomal protein components of the intercalated disc has been further demonstrated by 2 other studies linking the ACM phenotype to variants in the *CTNNA3*¹⁴ and *TJP1* genes.¹⁵

These initial discovery studies^{12,14,15} highlighted the potential contribution of components of the intercalated disc to the pathogenesis of ACM, thus widening its genetic basis. However, these findings require validation in large ACM patient cohorts to allow correct estimates of their relative contribution, prevalence, and clinical characteristics. To this end, we have collected the largest available worldwide population of previously genotype-negative ACM probands (n=500) to validate the contribution of *CDH2* variants to ACM and to establish a reliable prevalence. As a second step, we performed, whenever possible, cascade screening in the families of *CDH2* positive probands. Finally, to identify gene-specific features, we analyzed all *CDH2*-positive probands and affected family members from this and previously published studies,^{12,13} for a total of 9 probands and 15 family members.

METHODS

All data, analytical methods, and study materials supporting this study are available from the corresponding author on reasonable request for purposes of reproducing the results or replicating the procedures. The study was approved by the local Ethics institutional Review Boards, and the participants gave informed consent. A full description of the methods is provided in the [Data Supplement](#).

RESULTS

Study Population

Most of the 500 patients were White (n=416; 83%), followed by Han Chinese (n=77; 15%) and Black (n=2). In 5 cases, ethnicity was unknown. Males were 59% and females 41%.

Genetic Findings in *CDH2*

We identified 7 rare genetic variants (minor allele frequency <0.0001) in *CDH2*, 6 missense and 1 frameshift, in 8 unrelated subjects (Tables 1 and 2; Figure 1A). Among these, one variant was classified as pathogenic (p.D407N), 4 as likely pathogenic (p.P205L, p.N261D, p.N658Kfs*3, and p.P679A), and 2 as variants of unknown significance (Table 1). Cascade family screening was feasible in 4 *CDH2*-proband's families (p.P205L, p.N261D, p.D407N, and p.P679A), for a total of 12 subjects (7 variant positive and 5 variant negative). An additional family member, affected by ACM, died suddenly before genetic testing could be performed.

The p.D407N pathogenic variant, previously identified in 5 affected subjects of 2 independent studies,^{12,13} was also detected in 2/500 unrelated subjects comprising this study cohort. Both probands satisfied major ACM diagnostic criteria, such as structural dysfunction (important dilatation of RV) and depolarization/repolarization abnormalities (Table 2). One of the 2 p.D407N probands, symptomatic for arrhythmic events, had 3 affected relatives, one brother who died suddenly at age 18 years, another brother and a nephew (both carrying the p.D407N variant) with RV involvement and frequent premature ventricular complexes (PVCs) despite β -blocker therapy.

The p.N658Kfs*3 frameshift variant, resulting from a nucleotide duplication that putatively results in premature termination of the protein, is the first non missense variant thus far described in *CDH2*-associated ACM. The patient with this likely pathogenic variant is a female, diagnosed at 59 years, with dilatation of the RV, accompanied by depolarization/repolarization abnormalities and frequent PVCs (>500 per 24 hours, Table 2).

The other 3 variants classified as likely pathogenic (p.P205L, p.N261D, and p.P679A) are ultrarare missense variants (0 or 1 allele in gnomAD), located in the extracellular region of the protein (Figure 1A),

Table 1. Genetic Variants in the *CDH2* Gene Identified in the ACM Cohort

Genomic coordinates	Exon	Nucleotide change	Amino acid change	Conservation	Protein domain	MAF/allele count gnomAD	Variant classification	No. of probands	No. of positive FMs
chr18:25589769	5	c.614C>T	p.P205L	High	Cadherin 1 (EC1)	0.000003984/1	Likely pathogenic	1	2
chr18:25585879	6	c.781A>G	p.N261D	Full	Cadherin1-2 (EC1–EC2) interdomain	-	Likely pathogenic	1	2
chr18:25572744	9	c.1219G>A	p.D407N	Full	Cadherin 3 (EC3)	-	Pathogenic	2	2
chr18:25565494	12	c.1973dupA	p.N658Kfs*3	NA	Cadherin 5 (EC5)	-	Likely pathogenic	1	NA
chr18:25565138	13	c.2035C>G	p.P679A	Full	Cadherin 5 (EC5)	0.000003983/1	Likely pathogenic	1	1
chr18:25565036	13	c.2137G>C	p.D713H	Medium	Cadherin 5 (EC5)	0.00006365/16	VUS	1	NA
chr18:25565006	13	c.2167G>A	p.G723S	Full	Extracellular linker to transmembrane region	0.00003982/10	VUS	1	NA

Genomic coordinates are reported according to the GRCh37/hg19 assembly. Mutation nomenclature has been assigned according to the main transcript of the *CDH2* gene (NM_001792.4). Conservation has been assessed using the Vertebrate Multiz Alignment and Conservation (100 species) feature track of the UCSC genome browser (<https://genome.ucsc.edu>): Full=100% conserved; high = at least 95% conserved; and medium = at least 70% conserved. MAF/allele counts are reported considering the total population of the gnomAD database.¹⁶ Variant classification was made according to the ACMG guidelines.¹⁷ - indicates 0% frequency; ACMG, American College of Medical Genetics and Genomics; EC, extracellular cadherin ectodomain; FM, family member; MAF, minor allele frequency; NA, not applicable; and VUS, variant of unknown significance.

with evidence in support of pathogenicity coming also from genotype-phenotype correlation data in the respective patient families (Figure 2). The p.N261D variant affects the Ca²⁺-binding site in the EC1–EC2 (ectodomain 1–ectodomain 2) interdomain that plays a key role in cadherin-cadherin adhesion.²⁰ This variant was identified in a proband with sustained ventricular tachycardia (VT), implanted with an internal cardioverter-defibrillator at age 18 years, in one affected brother with left bundle branch block, non-sustained VTs and very frequent PVCs (40 000/24 hours), and in a cousin with signs of noncompaction and an episode of heart failure triggered by supra-VT (Figure 2). The p.P679A positive individual is a female who presented with syncope at age 20 years and who eventually developed biventricular structural impairment (Table 2). The variant was also present in her affected father, while it was absent in her unaffected sibling (Figure 2). The p.P205L variant resides in the EC1 domain that, together with EC2, is considered an essential structural element for cadherin-mediated intercellular adhesion.²¹ This variant was identified in a male proband who presented with repolarization abnormalities and nonsustained VTs. Cascade family screening also identified the variant in the affected father, while it was absent in 3 unaffected family members. A brother, positive for the same variant, was only partially investigated, and at present he cannot be clinically classified (Table 2; Figure 2).

Finally, 2 rare variants were classified as variants of unknown significance due to lack of a high degree of conservation of the affected residue, few clinical information available and the presence of the variant in exome/genome datasets, albeit with minor allele frequencies much lower than the disease prevalence (Table 1).²²

Pathogenic/likely pathogenic variants in other genes associated with arrhythmogenic and dilated cardiomyopathy were identified in only 3 probands not carrying any *CDH2* variant (Table I in the Data Supplement).

In conclusion, 7 rare variants in *CDH2* were identified in 8 out of 500 ACM unrelated patients (1.6% [95% CI, 0.5%–2.7%]) who lack a pathogenic/likely pathogenic variant in the major ACM genes. Considering only the 6 patients with American College of Medical Genetics and Genomics-graded pathogenic/likely pathogenic variants, the prevalence of *CDH2* clinically actionable variants in this ACM cohort is 1.2% (6/500; 95% CI, 0.2%–2.2%).

CDH2 Genetic Variability and Variant Location

Since *CDH2* is a novel human disease gene and to aid the eventual classification of genetic variants, it could be useful to evaluate its natural genetic variability. To this end, we took advantage of the publicly available exome/genome datasets including >140 000 subjects.

CDH2 is a gene extremely intolerant to loss-of-function variation, as indicated by the constraint metrics in the gnomAD database, with a high discrepancy between the number of expected (n=41) and observed (n=6) loss-of-function variants.

At the same time, the *CDH2* gene, as many other genes, seems to be more tolerant to missense variation, with 74% of the theoretically expected variants being actually observed in the gnomAD population.¹⁶ However, the number of subjects with rare (minor allele frequency <0.0001) and potentially clinically relevant missense variants differs significantly between the ACM cohort (pathogenic/likely pathogenic, 5/500, 1.0%) and the gnomAD population (potentially pathogenic, 300/140 000, 0.2%; *P*=0.0010). This difference becomes even more striking

Table 2. Task Force Diagnostic Criteria for ACM of the 6 Index Cases With Pathogenic/Likely Pathogenic Variants in CDH2

Proband (CDH2 variant)	Age at diagnosis	Sex	Ethnicity	Dysfunction and structural alterations		Repolarization abnormalities		Depolarization/conduction abnormalities		Arrhythmias		Family history	
				Major	Minor	Major	Minor	Major	Minor	Major	Minor	Major	Minor
Proband 1 (p.P205L)	34 y	M	Han Chinese	-	RVOT PLAX 30 mm, RVOT PSAX 34 mm. Regional RV dyskinesia (near RVOT)	Major: TWI V1-V4	Minor: -	Major: -	Minor: NSVT and >20000 PVCs per 24 h	Major: -	Minor: -	Major: -	Minor: -
Proband 2 (p.N261D)	17 y	M	White	Dilatation of RV, with aneurysm in RVOT. Fibrofatty replacement on endomyocardial biopsy	-	Negative TWs V1-V3	-	-	LBBB sustained VT, superior axis	Major: LBBB sustained VT, superior axis	Minor: 12000 PVCs	-	-
Proband 3 (p.D407N)	26 y	M	Unknown	Important dilatation of RV, hypokinetic, LVEF=45%, hypokinetic LV	-	Major: TWI V1-V3	Minor: -	Major: Ep-silon waves	Late potentials: QRS=143, LAS=74, RMS=3.6	Major: Electrical storms. Sustained VT, HR=210 bpm, LBBB pattern, axis =0°	Minor: -	Major: SCD at 18 y in a brother affected by ACM*	Minor: -
Proband 4 (p.D407N)	47 y	M	White	Severe impairment of RV function with mild LV dysfunction	-	Major: TWI V1-V6	Minor: -	Major: Ep-silon waves	-	Major: -	Minor: -	Major: -	Minor: -
Proband 5 (p.N658Kfs*3)	59 y	F	White	Dilatation of RV 118 mL/m ²	-	Major: TWI V1-V3	Minor: -	Major: Ep-silon waves	Late potentials	Major: -	Minor: PVCs>500 per 24 h - 4000 PVC/24 h	Major: -	Minor: -
Proband 6 (p.P679A)	20 y	F	White	Biventricular impairment (LVEF=44%; RVEF=34%)	-	Major: TWI V1-V4	Minor: -	Major: -	-	Major: -	Minor: 12688 PVCs on Holter. Presented with cardiac syncope. ICD implanted. ICD therapy for appropriate discharge for arrhythmia dL 155 ms.	Major: ACM in the father diagnosed via cascade screening	Minor: -

- indicates not present; ICD, internal cardioverter-defibrillator; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; NSVT, nonsustained ventricular tachycardia; PLAX, parasternal long axis; PSAX, parasternal short axis; PVC, premature ventricular complex; RVEF, right ventricular ejection fraction; RVOT, right ventricular outflow tract; SCD, sudden cardiac death; TW, T wave; TWI, T wave inversion; and VT, ventricular tachycardia.
*Genetic testing in the 18-y-old brother who died suddenly was not performed, due to lack of adequate material.

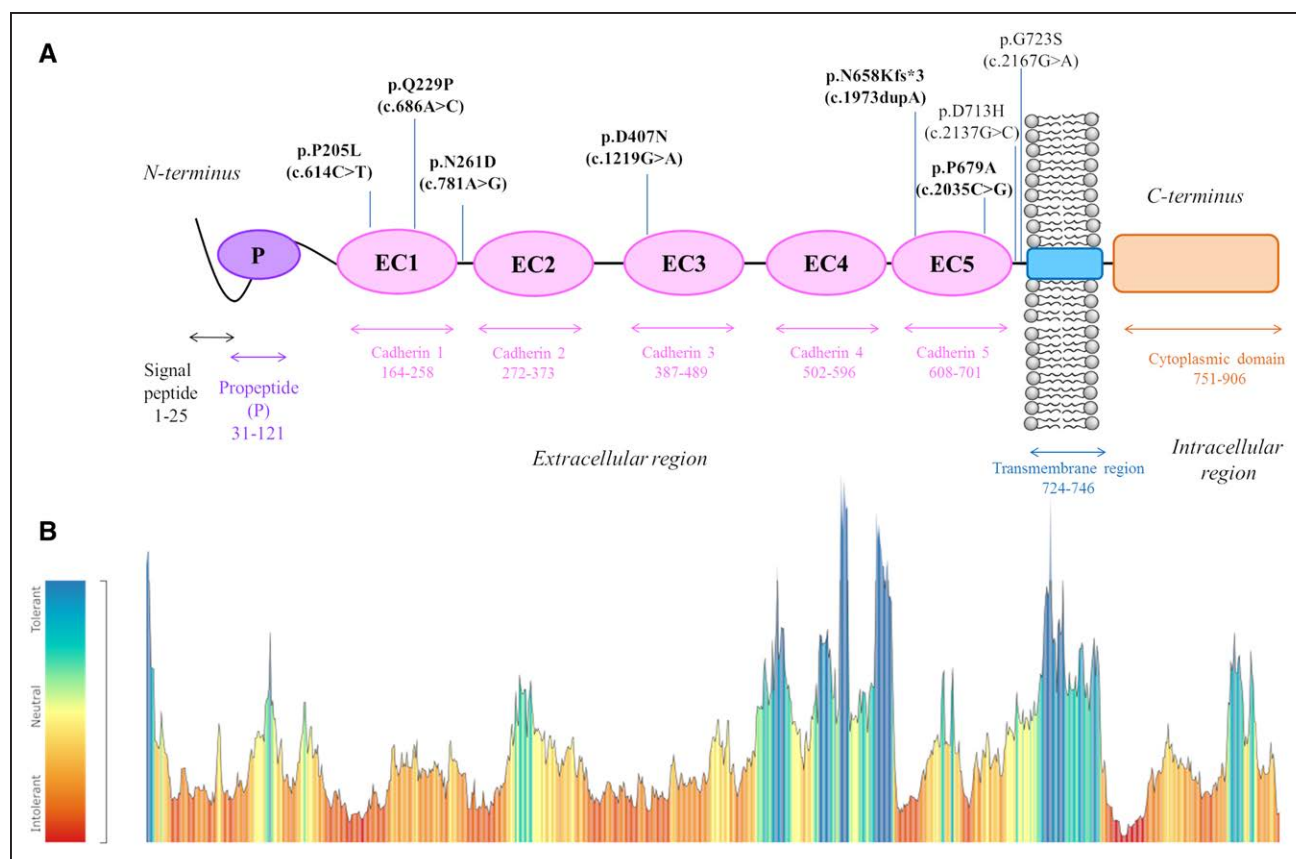


Figure 1. Schematic representation of the distinct domains of cadherin 2 protein and their respective genetic variant tolerance landscape.

A, The mature protein is formed by 5 extracellular repeats (EC1–EC5 [ectodomain 1–ectodomain 5]), a single-pass transmembrane region and a cytoplasmic C-terminal domain, while the N-terminal propeptide (P), following the signal peptide, is removed by proteolysis (Pfam database EMBL-EBI).¹⁸ The length/boundaries of each domain are indicated with double-headed arrows just below the protein. All arrhythmic cardiomyopathy (ACM)-associated *CDH2* genetic variants so far identified^{12,13} are depicted above the protein according to their position. Mutation nomenclature has been assigned according to the *CDH2* main transcript (NM_001792.4), with the amino acid change (p.) reported first and the corresponding nucleotide change (c.) in brackets. Pathogenic/likely pathogenic variants are shown in bold. **B**, *CDH2* tolerance landscape to missense variation, generated by MetaDome software analysis.¹⁹ The tolerance score is computed by the MetaDome software using the gnomAD population,¹⁶ as a missense over synonymous variant count ratio, calculated in a sliding window manner, for each position. The tolerance color-coded legend is reported on the left. The pathogenic/likely pathogenic missense variants identified in the ACM patient cohort all reside in red/orange regions (ie, the most intolerant to missense variation).

when considering only the ultrarare (minor allele frequency <0.000005) and potentially clinically relevant missense variants (pathogenic/likely pathogenic, 5/500, 1.0% in ACM cases versus potentially pathogenic, 59/140 000, 0.04% in gnomAD; $P < 0.0001$).

Regarding the localization within the protein of the pathogenic/likely pathogenic amino acid substitutions identified in our patient cohort, they exclusively reside in the extracellular region of cadherin 2 that appears intolerant to missense variation according to the computational analysis by MetaDome (Figure 1B).¹⁹ Specifically, most of these variants cluster in the head EC1–EC3 domains.

CDH2-Associated ACM Clinical Features

To identify gene-specific clinical features, we considered all probands with *CDH2* pathogenic and likely pathogenic variants of the present ($n=6$) and of the 2

previously published studies ($n=3$)^{12,13} along with their 15 affected family members with complete clinical information (7 from this and 8 from previously published studies), for a total of 24 patients with *CDH2*-associated ACM (Table 3).

Sustained VTs and sudden cardiac death occurred in 5/9 probands (56%). All except one of the remaining index cases had frequent PVCs and nonsustained VTs, with an internal cardioverter-defibrillator implanted in 6 (67%) (Table 3). The only proband with no spontaneous arrhythmias had VT induction at the EP study (electrophysiological) and recurrent episodes of heart failure. None of the other probands had heart failure episodes and none underwent a heart transplant during a mean follow-up period of 13.5 ± 8.5 years. Regarding the structural alterations observed, all probands had RV involvement, with a biventricular involvement observed in 3 (33%).

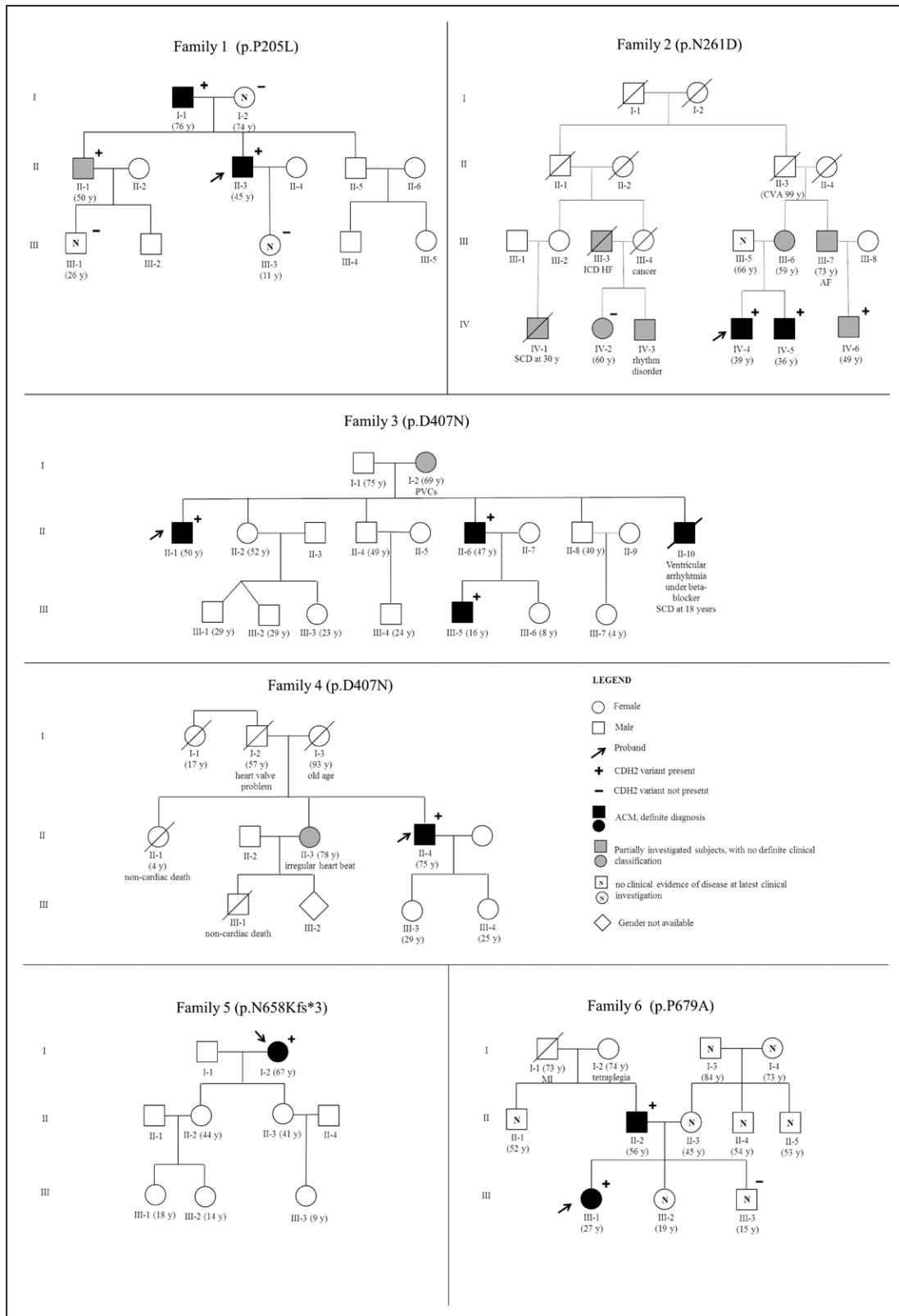


Figure 2. Pedigrees of the 6 families segregating the pathogenic/likely pathogenic variants identified in *CDH2*. The proband is indicated by an arrow. Black-filled symbols indicate affected subjects with a definite arrhythmogenic cardiomyopathy (ACM) diagnosis, while gray-colored symbols stand for partially investigated subjects for whom a complete clinical picture is not available. White symbols marked up with N correspond to subjects with no clinical evidence of disease at latest clinical investigation. + indicates mutation-positive subjects; – indicates mutation-negative subjects. CVA indicates cerebrovascular accident; HF, heart failure; MI, myocardial infarction; and PVC, premature ventricular contraction.

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Table 3. Arrhythmogenic Features of *CDH2*-Related ACM Patients so far Described

ACM patient	<i>CDH2</i> mutation	Proband/FM	Sustained VT/SCD	PVCs >500/24 h/NSVT	ICD	Outcome	Source
Family 1 (II-3)	p.P205L	Proband	-	+ (NSVT, PVCs)	-	Alive (45 y)	This study
Family 1 (I-1)	p.P205L	FM–Father	-	+ (NSVT, PVCs)	-	Alive (76 y)	This study
Family 2 (IV-4)	p.N261D	Proband	+ (sustained VT)	+ (PVCs)	+	Alive with ICD (39 y)	This study
Family 2 (IV-5)	p.N261D	FM–Brother	-	+ (NSVT, PVCs)	-	Alive (36 y)	This study
Family 2 (IV-6)	p.N261D	FM–Cousin	-	-	-	Alive (49 y)	This study
Family 3 (II-1)	p.D407N	Proband	+ (sustained VT)	-	+	Alive with ICD (50 y)	This study
Family 3 (II-6)	p.D407N	Brother	-	+ (PVCs)	-	Alive (47 y)	This study
Family 3 (III-5)	p.D407N	Nephew	-	+ (PVCs)	-	Alive (16 y)	This study
Family 3 (II-10)	NA	FM–Brother	+ (SCD)	NA	-	SCD at 18 y	This study
Family 4 (II-4)	p.D407N	Proband	-	-	+	Alive with ICD (75 y)	This study
Family 5 (I-2)	p.N658Kfs*3	Proband	-	+ (PVCs)	-	Alive (67 y)	This study
Family 6 (III-1)	p.P679A	Proband	-	+ (PVCs)	+	Alive (27 y)	This study
Family 6 (II-2)	p.P679A	FM–Father	-	+	-	Alive (56 y)	This study
Family 7 (ACM2 III-3)	p.Q229P	Proband	+ (sustained VT, SCD)	-	-	SCD at 24 y	12
Family 7 (ACM2 III-2)	NA	FM–Brother	+ (sustained VT, SCD)	-	-	SCD at 21 y	12
Family 7 (ACM2 III-4)	p.Q229P	FM–Cousin	-	+ (NSVT, PVCs)	-	Alive	12
Family 7 (ACM2 III-5)	p.Q229P	FM–Cousin	-	+ (PVCs)	-	Alive	12
Family 7 (ACM2 II-4)	p.Q229P	FM–Aunt	-	-	-	Alive	12
Family 7 (ACM2 II-7)	p.Q229P	FM–Aunt	-	-	-	Alive	12
Family 8 (ACM11 CM11.2)	p.D407N	Proband	+ (sustained VT)	-	+	Alive with ICD	12
Family 9 (III.2)	p.D407N	Proband	+ (BCE with appropriate ICD shocks)	+ (NSVT, PVCs)	+	Alive (21 y)	13
Family 9 (III.1)	p.D407N	FM–Sister	-	+ (NSVT, PVCs)	+	Alive (23 y)	13
Family 9 (III.3)	p.D407N	FM–Brother	-	+ (PVCs)	-	Alive (19 y)	13
Family 9 (II.2)	p.D407N	FM–Mother	-	+ (PVCs)	-	Alive (54 y)	13

In ACM patient column, the pedigree code used in the original study was reported as well in brackets. - indicates not present; BCE, breakthrough cardiac event; FM, family member; ICD, internal cardioverter-defibrillator; NA, not available; NSVT, nonsustained ventricular tachycardia; PVC, premature ventricular complex; SCD, sudden cardiac death; and VT, ventricular tachycardia.

Among the 15 clinically affected *CDH2*-positive family members, 2 suffered sudden cardiac death and 10 had frequent PVCs and nonsustained VTs (Table 3). Left ventricular involvement was present in 4 genotype-positive family members and in only one of them heart failure was reported during supra-VT.

DISCUSSION

The present report provides the first assessment of the prevalence of *CDH2* clinically actionable variants in ACM and establishes its potential clinical features. *CDH2* encodes cadherin 2, also known as N-cadherin, the main protein component of fascia adherens junctions at the intercalated disc. The main novel finding of this study is that pathogenic/likely pathogenic variants in *CDH2* are responsible for 1.2% of genetically elusive ACM cases (95% CI, 0.2%–2.2%). Second, the *CDH2*-p.D407N, found in 9 patients with ACM appears to be the most common variant associated with *CDH2*-mediated ACM.

In addition, this study shows the presence of 2 rather specific clinical features for this cohort of *CDH2*-ACM.

One is that left ventricular involvement was present in one-third of patients, with a rare evolution toward heart failure despite adequate follow-up period. The second is that the majority of *CDH2*-positive ACM patients have a ventricular arrhythmic phenotype (20/24, 83%), an observation with both prognostic and therapeutic implications.

Prevalence of *CDH2* Variants in ACM and Clinical Implications

After the initial discovery of *CDH2* variants as a novel genetic substrate of ACM,¹² a finding soon after replicated in another study,¹³ the need of estimating the contribution of *CDH2* variants to ACM pathogenesis in a large patient cohort emerged. Therefore, we have collected a cohort of 500 genetically negative ACM patients and performed genetic analysis of the *CDH2* gene. The prevalence of *CDH2* clinically actionable variants identified in this cohort is 1.2% (95% CI, 0.2%–2.2%), similar to our original estimation in the discovery study.¹² The yield of genetic testing in ACM is estimated to be close to

60%,^{23,24} and therefore, a significant number of patients remains genetically elusive. Although the prevalence of *CDH2* variants in ACM is low, this finding is not without potentially relevant clinical implications.

Indeed, the thorough clinical evaluation of those patients with *CDH2*-ACM enabled the identification of few gene-specific features. Specifically, some of the pathogenic variants so far identified, such as the p.Q229P¹² and the original p.D407N,^{12,13} seem to be associated with a severe arrhythmic phenotype, with recurrent sustained VT and sudden cardiac death. It seems reasonable to postulate that the sudden deaths which occurred in these families have been arrhythmic in nature also because all these cases were clinically affected by ACM. This is consistent with the fact that most of probands and affected family members with *CDH2* pathogenic/likely pathogenic variants had an arrhythmic phenotype (20/24, 83%), while the evolution toward heart failure appeared to be rare despite adequate follow-up.

These observations matter in terms of risk stratification and clinical management. Accordingly, *CDH2* genetic screening should be considered in patients with a definite diagnosis of ACM but without significant variants in major desmosomal genes. The identification of the disease-causing variant has clinical consequences for probands and family members alike. Given that the disease progression and arrhythmic risk for ACM patients are mostly linked to physical activity,¹⁵ the early identification of family members with subclinical or silent forms is important to establish preventive strategies that might have a favorable impact on the natural history of the disease.

Another issue to be considered is that if the concept that the *CDH2*-associated ACM has a predominantly arrhythmogenic profile will be validated—similar to other genetic forms of ACM, such as those linked to lamin A (*LMNA*), filamin C (*FLNC*), and phospholamban (*PLN*)⁴—specific recommendations for internal cardioverter-defibrillator implantation might ensue.²⁵ Clearly, all these observations are based on a limited number of patients. Further research and wider ascertainment strategies should provide more robust data for accurate genetic counseling of patients with *CDH2* variants.

Mechanistic Implications of *CDH2* Variants and Electrical Impairment in ACM

At present, the exact mechanism by which *CDH2* variants may affect cadherin 2 protein function and foster the clinical development of ACM is unknown; however, current knowledge allows discussing possible hypotheses.

The only non missense *CDH2* variant identified in this study, the p.N658Kfs*3, is expected to produce an aberrant mRNA transcript likely to be degraded by nonsense-mediated RNA decay²⁶ without being translated into protein, leading to loss-of-function by haploinsufficiency. The haploinsufficiency mechanism has been described

previously for truncating variants in proteins primarily involved in cell adhesion, such as the desmosomal cadherin desmocollin 2 (*DSC2*)²⁷ and other components of the desmosome.^{28,29} Cadherin 2 (or N-cadherin) haploinsufficiency, in particular, has been mimicked in vivo in a cardiac-specific mouse model (heterozygous conditional knockout) where it decreases connexin 43 plaque size leading to an increased susceptibility to ventricular arrhythmias.³⁰ Connexin 43 is the main structural component of gap junctions at the intercalated disc, and its reduction results in slow conduction, favoring arrhythmias by reentry.³¹ Furthermore, in these mice, also the expression of the zonula occludens 1, another protein of the intercalated disc recently implicated in ACM pathogenesis,¹⁵ was significantly reduced,³⁰ potentially concurring to the destabilization of gap junctions.

CDH2-encoded cadherin 2 (N-cadherin) is part of a protein cluster at the intercalated disc that also includes the cardiac sodium channel $\text{Na}_v1.5$ ³² and the ability of these 2 proteins to influence each other has been demonstrated in induced pluripotent stem cell-derived cardiomyocytes of a patient with ACM,³³ suggesting another potential mechanism of arrhythmogenesis.

The majority of pathogenic or likely pathogenic variants identified so far in *CDH2* are missense variants. A reasonable hypothesis for their mechanism of action could be that since they all reside in the extracellular EC domains of the protein (Figure 1), these variants may adversely affect normal adhesive function. Such a mechanism was recently demonstrated in vitro for *CDH2* missense variants associated to the agenesis of corpus callosum, axon pathfinding, cardiac, ocular, and genital defects syndrome, a multisystemic disorder characterized by developmental delay and intellectual disability, ocular and genital defects and congenital heart abnormalities.³⁴ In this condition, mutant cadherins with missense variants in EC4–EC5 showed a defect in cell-cell adhesion, with consequences both on self-binding and trans-binding with wild-type cadherin 2 proteins.³⁴ Another multisystemic disorder recently associated to genetic variants in *CDH2* is the Peters anomaly, mainly characterized by corneal abnormalities.³⁵ The reason why some *CDH2* variants may create these multisystemic disorders while others result in a pure ACM phenotype is currently unknown; further studies are needed to unravel the functional effect of specific *CDH2* variants and the molecular mechanisms leading from cadherin 2 dysfunction to different phenotypes.

Limitations

One limitation, intrinsic to the nature of collection of data from multiple sources, is the limited number of family members that could favor an ascertainment bias towards the more severe end of the spectrum of the disease. The family members effectively evaluated (*CDH2*-variant

positive, with available clinical data) are only part of all other potential carriers. The evaluation of further *CDH2*-positive patients should reflect more precisely the different phenotypic variability present in this disease subtype and may modulate the severity of disease or the specific clinical features emerged so far.

This implies that the severity of *CDH2* variants, as well as their association with other cardiac phenotypes beyond ACM, may need to be refined once a greater number of positive patients will be available.

Conclusions

The contribution of *CDH2* clinically actionable variants to ACM is small but significant. Indeed, this rare genetic form appears to be associated in our cohort with a high arrhythmogenic profile and, therefore, its evaluation may be reasonable in patients affected by ACM in whom a disease-causing variant has not been identified. Whether or not the identification of disease-causing variants in this gene should modify the management of an otherwise still asymptomatic patient, as already happens for other rare genetic subtypes, such as *LMNA* and *FLNC*, remains to be determined.

We recognize the inherent ascertainment biases present in this type of study; however, current data suggest that there is a high incidence of ventricular arrhythmias in *CDH2*-ACM patients, with few evolving to heart failure.

ARTICLE INFORMATION

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Supplemental Materials

Online Materials and Methods
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