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Integrative Genomic and Literature Assessment of Desmoglein 2 Related Arrhythmogenic Cardiomyopathy with Italian Cohort Validation

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Abstract

Background. Desmoglein-2 (*DSG2*) is an essential cardiac desmosomal cadherin, and its alteration underlies a broad spectrum of arrhythmogenic cardiomyopathy (ACM). Yet, the clinical significance of many *DSG2* variants remains uncertain. This study aimed to systematically characterize the spectrum, structural impact, and clinical relevance of *DSG2* variants by integrating large-scale genomic evidence, published data, and a deeply phenotyped validation cohort.

Methods. We conducted a systematic literature review (115 studies; 145 curated variants) and analyzed population-scale datasets (3,570 variants in gnomAD; 1,847 in ClinVar). All variants were uniformly reclassified following ACMG/ClinGen criteria. A validation cohort of 95 Italian *DSG2*-carriers underwent detailed phenotyping. Structural modelling via AlphaFold, supported protein modelling, calcium-binding site prediction, and DynaMut stability analysis were performed to evaluate the functional consequences of key variants.

Results. Literature and database integration reveal domain-specific variant clustering, with high-impact missense variants enriched in calcium-binding extracellular domains, the furin cleavage site, and the intracellular PKP2-binding region. In the validation cohort, penetrance among genotype-positive relatives is 42%, while 13% of definite ACM cases experience major ventricular arrhythmias; transplantation and mortality each occur in 3%. Biallelic and digenic variants are associated with earlier onset and more severe biventricular involvement. Structural modelling confirms that pathogenic missense substitutions destabilize DSG2 architecture or impair calcium-dependent adhesion.

Conclusions. This study refines the classification of *DSG2* variants and highlights the importance of domain-level and multilocus interpretation in ACM. These findings support comprehensive genetic screening, structural modelling for variant assessment, and lifelong follow-up of *DSG2* carriers to improve diagnosis and risk stratification.

Plain Language Summary

Arrhythmogenic cardiomyopathy (ACM) is an inherited heart disease that can cause abnormal rhythms and sudden cardiac death, often in young adults. This study focused on the *DSG2* gene, which helps heart cells stick together. We combined genetic data from large population databases, published studies, and a group of Italian patients with ACM to understand how different *DSG2* changes affect disease risk. We found that disease severity depends not only on the type of genetic change but also on the location within the gene and whether other related genes are involved. These findings improve understanding of inherited heart disease and support more accurate genetic testing and follow-up for affected families.

Introduction

Desmoglein (DSG) is a transmembrane glycoprotein belonging to the cadherin superfamily of calcium-dependent adhesion molecules. Alongside desmocollin (DSC), another cadherin superfamily member, desmoglein assembles at the plasma membrane to form adhesive intercellular structures known as desmosomes. These structures are particularly important for providing adhesive strength in tissues that are continuously exposed to mechanical stress, such as the myocardium, bladder, and epithelia [1]. A high-resolution model of desmosome architecture, developed by integrating cryo-electron tomography and molecular dynamics, has demonstrated that desmosomal cadherins, DSGs and DSCs, extend from the membranes of opposing cells and interact at a dense midline, forming a sieve-like structure [2].

Specifically, DSGs and DSCs form calcium-dependent homophilic (i.e, DSC-DSC and DSG-DSG) and heterophilic (i.e, DSC-DSG) interactions between adjacent cells through their extracellular (EC) domains, while their cytoplasmic tails associate with armadillo proteins (plakoglobin, JUP and plakophilin, PKP2) and desmoplakin (DSP), which anchor these complexes to the intermediate filaments of the cytoskeleton. This integrated system endows desmosomes with the ability to maintain cellular cohesion and withstand mechanical stress in the epithelial and the myocardial tissues [3].

In humans, four isoforms of the DSG protein (DSG1-4) are expressed in tissue-specific patterns corresponding to differentiation stages, likely influencing their adhesive properties. While all DSG isoforms are present in the epidermis, DSG2 (MIM#610193) is the only isoform expressed also in cardiac myocytes. Consequently, any loss of DSG2 function in the myocardium cannot be compensated by other DSG isoforms [4].

The mature DSG2 protein has a molecular weight of 117 kDa and features a pro-peptide region (Pro) followed by four highly conserved extracellular cadherin homology domains (ECs 1-4), which contain calcium-binding motifs. These are followed by an extracellular anchor (EA), a single transmembrane domain (TM), and an intracellular anchor (IA). The intracellular portion of DSG2 consists of a cadherin-specific intracellular segment (ICS) that interacts with plakoglobin (*JUP*), a proline-rich linker (LD), six repeat unit domains (RUDs), and a glycine-rich terminal domain (TD) [5, 6].

Both extracellular and intracellular domains of DSG2 are known targets of matrix metalloproteinases, cysteine proteases, and lectins [7]. This proteolytic activity is thought to play a key role in modulating DSG2 adhesive and non-adhesive functions, including proliferation, differentiation, tissue morphogenesis, cellular sorting, and apoptosis [8, 9]. Additionally, *DSG2* dysregulation has been increasingly associated with epithelial cancer progression, as it disrupts cell adhesion and promotes invasion, metastasis, and epithelial-to-mesenchymal transition (EMT) [10, 11].

Due to its essential function in preserving desmosomal integrity within the myocardium, *DSG2* remains one of the highest-ranked genes with definitive evidence for its causative role in arrhythmogenic cardiomyopathy (ACM) classic variant [12, 13], despite detailed clinical characterizations remain limited. To date, numerous *DSG2* variants including missense, nonsense, and splice-site variants have been deposited in publicly accessible variant databases such as Genome Aggregation Database (gnomAD) and ClinVar. However, the clinical significance of many of these variants remains uncertain, highlighting the need for ongoing functional studies, genotype–phenotype correlations, and segregation analyses to better understand their pathogenic potential.

In this study, we integrate systematic literature review, curated population-scale datasets, and a deeply phenotyped Italian validation cohort to comprehensively assess *DSG2* variants in ACM. By mapping variants across structural domains and applying current reclassification criteria, we refine *DSG2* variant interpretation and identify domain-specific mutational hotspots, providing a framework for improved molecular diagnosis and precision risk assessment in ACM.

Methods

Literature Search

Systematic searches were conducted on PubMed using sequential key strings: "Desmoglein 2," "Desmoglein 2 AND cardiomyopathy," "Desmoglein 2 AND cardiomyopathy AND mutation(s)," "Desmoglein 2 AND cardiomyopathy AND genetic variant(s),"and "Desmoglein 2 AND cardiomyopathy AND desmoglein 2 mutation(s) OR desmoglein 2 variant(s)". No restrictions were applied regarding publication dates. Studies were included if they were original articles written in English, with titles and abstracts relevant to the topic. The reference lists of

included studies were also analyzed. Exclusion criteria comprised articles written in other languages, reviews, retracted publications, duplicates, or those with irrelevant titles and abstracts.

Database Analysis

DSG2 variants were systematically collected from the gnomAD v4.1.0 and ClinVar databases. GnomAD provided insights into allele frequencies across diverse populations, while ClinVar offered curated annotations of clinical significance. This integration allowed for a comprehensive assessment of *DSG2* variant distribution, population frequencies, and disease associations.

Validation of the Padua Cohort

The study cohort comprised unrelated *DSG2*-positive index patients diagnosed with ACM according to the revised 2010 Task Force Criteria [14], as well as their *DSG2*-positive relatives. The 2020 Padua Criteria [15] were applied to enhance diagnostic specificity, allowing precise phenotypic classification of *DSG2*-related ACM, particularly in distinguishing right-dominant, left-dominant, and biventricular forms.

For inclusion in the validation cohort, only variants classified as LP/P, and VUS, were considered. The study was approved by Ethics Committee for Clinical Research of the Azienda Ospedale Università Padova, and all participants provided written informed consent

Genetic Screening

DNA was extracted from whole blood using the MagNA Pure Compact System (Roche, Germany). Quality and concentration were assessed using a Qubit fluorometer, NanoDrop spectrophotometer, and TapeStation 4200. Targeted Next-Generation Sequencing (NGS) was performed with the TruSight Cardio Sequencing Kit (Illumina, USA), covering 174 genes related to inherited cardiac conditions, including *DSG2* (NM_001943.4). Variants passing quality and frequency filters were confirmed by Sanger sequencing. CNVs were analyzed via multiplex ligation-dependent probe amplification (MLPA), using the SALSA MLPA P168 ARVC-*PKP2* probe mix (MRC-Holland). Ratios indicative of deletions (<0.6) or duplications (>1.4) were confirmed through repeated experiments.

Variant Classification

Single nucleotide *DSG2* variants were classified based on ACMG guidelines (2015) and 2018 cardiomyopathy-specific recommendations, whereas CNVs according to the ACMG and ClinGen joint recommendations (2020) [16, 17, 18]. Specifically:

- **Frequency thresholds:** gnomAD MAF < 0.000036, as defined by the ClinGen Reappraisal of Genes Associated With Arrhythmogenic Right Ventricular Cardiomyopathy [13].
- **Databases used:** gnomAD v4.1 and ClinVar.
- **Computational algorithms (PP3/BP4/BP7)** were applied in accordance with ACMG/AMP guidelines for sequence variant interpretation, which refined the use of in silico predictors [19,20]. Four independent tools were used: BayesDel, MutPred2, REVEL and VEST4. PP3 or BP4 was assigned when ≥3 of the 4 algorithms yielded concordant deleterious or benign predictions, respectively. BP7 was applied to synonymous variants with no predicted splice impact and high conservation concordance across tools.
- **The BP1** criterion was not applied in order to avoid bias in the classification of missense variants.
- **Definition of hotspots for PM1:** extracellular calcium-binding domains EC1–EC4, furin cleavage site, PKP2-binding site.
- **Criteria for downgrading PVS1:** last-exon variants considered less impactful due to likely NMD escape.
- **Treatment of "reputable source" evidence:** either ClinVar submissions from expert panels or records supported by functional data and segregation analysis.
- In a 2020 recommendation, the ClinGen Sequence Variant Interpretation (SVI) Working Group proposed that criterion PM2 (absence or rarity in population databases) should, by default, be applied at supporting rather than moderate strength (SVI Recommendation for Absence/Rarity (PM2) – Version 1.0). The ACGS Best Practice Guidelines for Variant Classification in Rare Disease (2024), however, continue to permit PM2_moderate according to the original ACMG framework, pending formal harmonization of the criteria. A recent study [21] further demonstrated that PM2 strength may vary with the prior probability of pathogenicity and allele frequency context.

Accordingly, in this study, we applied PM2 at moderate strength, consistent with current ACMG/AMP and ACGS practice, while awaiting the release of updated official ClinGen recommendations

Clinical data collection.

ECG, echocardiography, and CMR evaluations were performed according to current ESC 2023 Guidelines [22].

Estimated Penetrance

To assess the penetrance of *DSG2* variants within affected families, we calculated the proportion of genotype-positive individuals who exhibited a clinical phenotype (phen +), excluding probands to avoid ascertainment bias. Penetrance was defined as the number of individuals expressing the phenotype divided by the total number of genotype-positive individuals within a family. We compared carriers within our cohort to data from published studies, that met our inclusion criteria and focused on family co-segregation analyses. The penetrance formula applied in both contexts was:

$$Penetrance\ (\%)\ (Number\ of\ phenotype-positive\ individuals\ /\ Number\ of\ genotype-positive\ individuals) \times 100$$

Bioinformatic Analysis

To assess the potential functional impact of *DSG2* missense variants, we performed a multi-step structural modelling analysis integrated directly into the variant classification workflow. First, the full-length DSG2 structure was obtained using AlphaFold (AF-Q14126-F1-model_v4) [23], which provides high-confidence modelling of extracellular cadherin domains, the furin-cleavage region, the transmembrane helix, and the intracellular armadillo-binding segments. Variant-specific models were generated by introducing amino acid substitutions into the AlphaFold reference structure.

Calcium-binding residues and coordinating motifs within EC1–EC4 were annotated using IonCom [24], which predicts metal-binding sites based on evolutionary conservation, physicochemical parameters, and structural templates. This allowed identification of variants that may disrupt Ca^{2+} coordination, a critical requirement for cadherin rigidity and adhesive function.

To evaluate the energetic and conformational effects of individual substitutions, we employed DynaMut [25], which computes predicted changes in protein stability ($\Delta\Delta G$), alterations in local vibrational entropy, and perturbations of intermolecular interfaces. Variants predicted to markedly destabilize the EC domains, interfere with calcium-binding geometry, or disrupt the PKP2-binding region in the intracellular cadherin-like segment (ICS) were flagged as structurally high-impact.

These structural outputs were incorporated as supportive evidence during ACMG/ClinGen classification. Specifically, destabilizing or functionally disruptive predictions from two or more independent tools (AlphaFold context + IonCom + DynaMut) contributed to PP3 (computational support for a deleterious effect), while variants with stable structural predictions and preserved Ca^{2+} coordination contributed to BP4/BP7 where applicable. Structural modelling also guided the definition of PM1 hotspots by validating the functional relevance of conserved calcium-binding loops, the furin cleavage motif, and the PKP2-interacting domain. [26]

This integrated modelling framework ensured that structural predictions were not treated as a separate exploratory analysis but were systematically embedded within the variant interpretation pipeline and directly influenced final classification when concordant with other ACMG parameters.

Results

Genetic Variants overview

Literature. Since *DSG2* was first implicated in ACM in 2006 [12], extensive efforts have catalogued its genetic variability, revealing a broad phenotypic spectrum. Analysis of 115 literature studies (Supplementary Data S1) and 145 variants (Supplementary Data S2) using current ACMG criteria identified 103 variants as either likely pathogenic or pathogenic (LP/P) or variants of uncertain significance (VUS). Among them, 60 were missense variants and 43 were radical variants, i.e., determining a severe or disruptive effect, such as frameshift, nonsense, splice-affecting, or large structural changes. Notably, 18% of missense variants were classified as 'hot-VUS' (4–5 ACMG points) predominantly within extracellular domains (EC1-EA), while 24% were LP/P variants clustered in the pro-peptide and EA regions (Figure 1A). High impact missense variants, predicted or shown to have a strong disruptive effect on the structure or function of the protein, were reported to alter the conserved furin recognition sequence (Arg-Gln-Lys-Arg; residues 46–49) [27, 28] located in the Pro (Figure 1B), as well as those affecting the cytoplasmic PKP2-binding site [29] (Figure 1C). Regarding radical variants, 84% were classified as LP/P (Figure 2A).

Databases. As of this study, the gnomAD database lists 3,570 *DSG2* variants, with nearly half being missense but only ~1% (n.15) classified as LP/P, whereas loss-of-function (LOF) variants more frequently obtained LP/P classification predominantly located in extracellular domains (ECs 1-4, 28%, n.39) and cytoplasmic domains (21%, n.29, primarily RUD) (Figure 2B). Similarly, ClinVar reports 1,847 *DSG2* variants, nearly half missense categorized as benign/likely benign (B/LB) and VUS, with only 17 (2%) missense variants deemed LP/P, highlighting a critical need for functional validation (Figure 2C). In contrast, LOF variants are more frequently classified as LP/P (https://search.clinicalgenome.org/CCID:007037).

Padua Cohort. In the Padua validation cohort of 95 ACM patients, including 46 probands (48%), 31 heterozygous *DSG2* variants were identified, including 15 radical and 16 missense variants, primarily affecting EC domains (Figure 2D, Supplementary Data S3). Some of probands (6.5%) had a family history of ACM, with symptom onset averaging 37 ± 17 years and male predominance (55%). Phenotypic characterization based on 2010 Task Force Criteria showed 56.4% definite ACM diagnosis, 5 (5.3%) for a borderline diagnosis, and 36 (38.3%) for a possible diagnosis. During a 6.6 ± 8.2-year follow-up, 13% of definite ACM patients experienced major ventricular

arrhythmias (MVA), in alignment with the estimated risk of arrhythmic events by the arrhythmogenic right ventricular cardiomyopathy (ARVC) Risk Calculator which was 10% at 5 years, 3% required transplantation, and 3% died (Table 1). Of note, no MVA were detected among the borderline/possible ACM groups. Histopathologic findings from a transplanted heart and a sudden cardiac death (SCD) case are illustrated in Figure 3.

Penetrance of *DSG2* variants was 42% (10 phenotype positive out of 24 genotype positive relative carriers) in our cohort, slightly below the 52% (38 phen + out of 72 carriers) reported in broader literature (18 published studies which collectively included 72 genotype-positive family members). (see Supplementary Data S1 for reference details). A detailed clinical and instrumental analysis is presented in Table 1.

Table 1. Detailed clinical and instrumental analysis of *DSG2* carriers.

Parameters	**Overall n=95 (100 %*DSG2* carriers)**
Age at symptoms onset (years)	37 ±17
Male gender	52 (55)
Proband status	46 (48)
ICD implantation	24 (25)
Genetics	**Available in 95 carriers (100 % *DSG2* carriers)**
Heterozygous	79 (83)
Homozygous	4 (4)
Compound heterozygous	9 (9)
CNVs	3 (3)
Family History	**Available in 46 probands (48 % *DSG2* carriers)**
Probands with family history of SCD or Cardiac Arrest	11 (24)
Arrhythmogenic Cardiomyopathy Classification (2020 Padua Criteria)	**Available in 46 probands (48 % *DSG2* carriers)**
Right dominant	15 (32)
Left dominant	8 (18)
Biventricular	23 (50)
Electrocardiographic features	**Available in 62 carriers (65 % *DSG2* carriers)**
Negative T waves v1-v3	31 (50)
Negative T waves v4-v6	17 (27)
Low precordial QRS voltages	14 (22)
Echocardiographic characteristics	**Available in 62 carriers (65 % *DSG2* carriers)**
Left ventricular dilatation	10 (16)
Left ventricular ejection fraction	58 ± 8
Right ventricular dilatation	33 (53)
Right ventricular fractional area change	37 ±11
Right ventricular akinesia/dyskinesia	35 (56)
Cardiovascular Magnetic Resonance characteristics	**Available in 34 carriers (35 % *DSG2* carriers)**
Fatty infiltration	20 (59)
Non-ischemic fibrous replacement	30 (88)
Major Adverse Cardiovascular Outcomes	**Available in 62 carriers (65 % *DSG2* carriers)**
Major ventricular arrhythmias	8 (13)
Cardiac Transplant	2 (3)
Death	2 (3)

Values are given as nr. (% total analyzed) or mean ± SD unless otherwise indicated.

Biallelic *DSG2* variants: homozygous and compound/double heterozygous

Although *DSG2* variants are traditionally associated with autosomal dominant inheritance in ACM, emerging evidence underscores a more severe disease phenotype in individuals with biallelic alterations, supporting a "second-hit" or dose-dependent model. Heterozygous carriers frequently remain asymptomatic or display only mild phenotypes, whereas homozygous or compound/double heterozygous individuals tend to exhibit early-onset and aggressive disease forms.

Literature. Recessive inheritance was first reported by Rasmussen et al. in a family harboring the *DSG2* c.1003A>G p.(Thr335Ala) variant (overall MAF 0.000707 - European Finnish: MAF 0.00112. No homozygous individuals reported in gnomAD) [27], localized in the EC3 domain, and later confirmed in another unrelated family [30]. In both reports, homozygous carriers presented with severe ACM in the absence of dermatological features, while heterozygous relatives remained unaffected. This variant has since been identified in both affected individuals and healthy controls [31]. Similarly, the EA domain variant c.1592T>G p.(Phe531Cys) has been described as a founder mutation in East Asian populations (overall MAF 0.000015 - East Asian: MAF 0.00052. No homozygous carriers reported in gnomAD) [32, 33], further supporting the pathogenic role of biallelic missense variants, particularly in EC domains (Supplementary Data S2). Notably, homozygous variants represent approximately 5% of all *DSG2* variants reported in the literature, highlighting their relative rarity but potential clinical relevance.

Padua Cohort. Within the Padua validation cohort, 4 *DSG2* variants were detected in homozygous form, each in different proband (4%) (mean age 17 ± 6.5 years; 75% female). Of these variants, 3 were also observed in other probands or relatives in the heterozygous state. One female proband carrying the homozygous c.1003 A>G p.(Thr335Ala) variant demonstrated early symptom onset at age 25 with palpitations, frequent PVCs, and a suspected biventricular ACM phenotype without ventricular dysfunction or major adverse cardiovascular events (MACE). Both parents of the proband were identified as heterozygous carriers of the *DSG2* c.1003 A>G variant but, clinically, they remained asymptomatic. In the father, CMR imaging revealed a small focus of fibrosis and regional hypokinesia, and 24-hour Holter monitoring documented frequent ventricular premature beats. Despite these findings, neither parent currently meets the diagnostic criteria for ACM. Another case involved a homozygous Tunisian child with the *DSG2* c.2410del p.(Thr804Leufs*4),

extremely rare and absent in gnomAD, presenting with right-dominant ACM at age 11, still free of MACE, with frequent isolated PVCs on Holter monitoring; both heterozygous parents were asymptomatic and lacked major diagnostic criteria. (Supplementary Data S3). Last, a 38-year-old woman homozygous for *DSG2* c.1672C>T p.(Gln558*) developed biventricular dysfunction and underwent heart transplant but died from complications. The explanted heart (440 g, excluding atria) showed RV aneurysms (inferior sub-tricuspid and apical) and fibrofatty replacement; similar replacement was noted in the right side of the septum and subepicardial posterolateral LV wall, with focal inflammation and calcifications (Figure 3). The heterozygous carrier presented with RV involvement only and maintained a more preserved LV systolic function compared with the homozygous carrier, suggesting a less severe expression of the disease. Up to 30% of disease-causing *DSG2* alleles harbor premature stop codons, either through nonsense or frameshift variants [34]. Most of these are expected to trigger nonsense-mediated mRNA decay (NMD) when the premature termination codon occurs more than 50 bp upstream of the final exon–exon junction. However, truncating variants located in the terminal exons, such as c.2410delA and likely also c.1672C>T (p.Gln558*), may escape NMD, leading to the production of partially functional truncated proteins. This residual protein expression could explain the survival of homozygous carriers and the variable disease severity observed. In contrast, complete *DSG2* knockout models in mice result in embryonic lethality, supporting that full loss of *DSG2* function is incompatible with life [35]. These observations suggest that both human variants likely retain limited desmosomal activity, preventing complete functional loss.

Compound heterozygosity in *DSG2*, observed in 8.5% of the distinct *DSG2* variants reported in the literature with at least one case described in a compound heterozygous state, has similarly been linked to severe ACM phenotypes [36, 37, 38] (see Supplementary Data S4). Six individuals from the Padua cohort (5 males, mean age 44 ± 26 years; 8%) carried compound/double heterozygous *DSG2* variants. In one male proband carrier, two missense variants *in trans* (compound*)* configuration, p.(Ala131Thr) and p.(Ala358Thr), affecting the EC1 and EC3 domains, respectively, resulted in early onset at age 13 and progression to biventricular disease with fibrofatty LV involvement and ICD implantation. Another case featured a female proband with *DSG2* c.689A>G, p.(Glu230Gly) and c.890A>G, p.(Asp297Gly) variants in EC2 and EC3, respectively, affecting calcium-binding

residues essential for cadherin function (Figure 4) . The proband presented with sustained ventricular tachycardia and developed severe RV dysfunction, requiring ICD protection. Additionally, the remaining 4 probands were found to carry *DSG2* c.298G>C p.(Gly100Arg) and c.3059_3062delAGAG p.(Glu1020Alafs*18) variants *in cis* (double) configuration. Three exhibited biventricular ACM with late gadolinium enhancement (LGE) on CMR without MACE, while the last proband had a mild phenotype and several family members of all 4 families were unaffected, indicating incomplete penetrance and the need for more genotype-phenotype correlation studies (see Supplementary Data S3, Figure 5).

DSG2 founder variant

Literature. Founder variants originate in a single individual or small group and are passed through generations, often becoming more common in isolated populations due to genetic drift, limited gene flow, and population bottlenecks. These variants are particularly significant in specific communities, where they can increase the prevalence of inherited disorders [39]. Unlike in North America and Europe, where *DSG2* variants occur in 5–10% of ACM probands, they are more common in East Asia due to founder effects leading to homozygosity. The *DSG2* c.1592 T>G p.(Phe531Cys) variant was the first globally recognized *DSG2* founder variant, reported in East Asian ACM patients in 2018 [40, 41]. It was found in eight homozygous probands with full ACM phenotype penetrance, including early-onset biventricular involvement. Heterozygous carriers were mostly asymptomatic or showed mild symptoms in 25% of cases. Haplotype analysis confirmed its founder status, with an allele frequency of 0.12% in East Asia and 8.47% in the Chinese ARVC cohort [33]. A Japanese ACM case involving this founder variant was also documented [42].

DSG2 de novo variants

Literature. *De novo* variants in desmosomal genes associated with ACM are exceptionally rare, with only 1.4% identified in a multinational study of 322 ACM patients [43]. The first *DSG2 de novo* variant was reported in 2009, and to date, only two cases have been fully documented (1% of all *DSG2* variants reported in literature). Both cases involved unremarkable family histories, normal cardiac evaluations of relatives, and genetic testing confirming the variants were not inherited from either parent or siblings (with confirmed paternity). Further details of these variants are provided in Supplementary Data S2.

DSG2 copy number variations

Literature. Copy number variations (CNVs), which involve large-scale deletions or duplications of genomic DNA, are increasingly recognized as important contributors to inherited cardiac diseases such as ACM. These structural alterations can disrupt gene dosage, interfere with regulatory elements, or act synergistically with point mutations to influence disease expression. ClinVar lists 31 large CNVs encompassing *DSG2* among other genes, most associated with

phenotypes outside the classical ACM spectrum. This may in part reflect underrecognition of ACM in individuals with neurodevelopmental or congenital anomalies who are less likely to undergo cardiologic evaluation as first clinical examination. However, CNVs in ACM remain underexplored due to the technical complexity of their detection, with only two systematic studies reported to date.

Padua Cohort. Our group previously identified a pathogenic CNV in a cohort of 160 ACM probands lacking point mutations in desmosomal genes. This CNV was a 482 Kb heterozygous deletion on chromosome 18q (chr18:31065974–31549007, GRCh38/hg38) encompassing both *DSG2* and *DSC2*, detected in a 15-year-old patient presenting with negative T waves on precordial ECG during sports screening and a strong family history of SCD. The disease rapidly progressed to severe biventricular dysfunction with fibro-fatty replacement, necessitating prophylactic ICD implantation [44].

In the current cohort, we identified another clinically relevant CNV involving a 45-year-old male carrying a duplication of the same chromosomal region (chr18:31102163-31546313, GRCh38/hg38) affecting both *DSG2* and *DSC2*. Initially diagnosed with dilated cardiomyopathy (DCM) after a cardiac arrest at age 36, ACM was confirmed via endomyocardial biopsy. The patient experienced multiple arrhythmic episodes and was eventually hospitalized for heart failure. His family history was notable for SCD in his mother (age 37), sister (age 35, postpartum), and brother (age 54), while his 16-year-old son, diagnosed with ACM after exercise-induced extrasystoles, further highlighted the variants penetrance and clinical relevance.

Supporting these observations, another study described a case of compound heterozygosity involving a large *DSG2* deletion *in trans* configuration with a nonsense variant [45], reinforcing the notion that CNVs affecting desmosomal cadherins can significantly modulate disease phenotype and severity. These findings emphasize the need to include CNV screening in the genetic evaluation of ACM, especially in genetically.

DSG2 digenic variants

Literature. Digenic inheritance further broadens the complexity of ACM pathogenesis. Studies show that up to 5% of ACM cases harbor digenic mutations (e.g., *DSG2* and *PKP2*), correlating with earlier onset and increased severity [46]. However, when considering specifically digenic

combinations involving *DSG2*, their prevalence rises to as much as 14% of all reported cases in the literature. Notably, individuals carrying both DSG2 p.(Arg46Gln) and rare *DSP* variants -e.g., p.(Val30Met or p.(Asn593Ser)- exhibited full ACM phenotypes, while single heterozygous carriers often remained unaffected [31]. These findings highlight the synergistic impact of multiple desmosomal gene variants and underscore the importance of comprehensive genetic screening. A full summary of compound and digenic variants reported in the literature is presented is presented in Supplementary Data S2 and S5.

Beyond radical genetic variants

In a study aimed at characterizing the expression profile of desmosomal proteins in explanted ARVD/C heart samples, Vite et al. investigated the observed decrease in the mRNA levels of desmosomal cadherins (*DSG2* and *DSC2*) and proposed that increased degradation or a translation defect, rather than mRNA downregulation, could be the underlying cause [47].

Not only radical variants, but also polymorphisms in *DSG2*, may affect protein levels. Specifically, a 2-bp insertion-deletion (indel) polymorphism in the 3' UTR of *DSG2* (rs397729601) has been associated with reduced *DSG2* expression by disrupting the binding of miR-933-3p to *DSG2*, which may contribute to SCD [48].

Discussion

Despite its established role as the third major gene implicated in ACM, detailed clinical characterizations of *DSG2*-related ACM remain limited. The phenotype typically mirrors classical ARVC, with hallmark features such as fibrofatty myocardial replacement, desmosomal remodeling, precordial T-wave inversions, frequent PVCs, and VT with left bundle branch block (LBBB) morphology [12, 49, 50, 51]. These features are reminiscent of those observed in carriers of *PKP2* radical variants, underscoring shared arrhythmic risk profiles between the two genotypes.

However, emerging data suggest *DSG2* variants may predispose to a more complex phenotype. While arrhythmic risk is comparable to *PKP2*-associated disease, *DSG2* carriers, particularly those with specific variants, show an increased tendency toward LV dysfunction and progression to heart failure [52, 53]. Although *DSG2* has not been definitively linked to isolated LV cardiomyopathy [54], some pathogenic variants -e.g., p.(Thr335Ala)- have been described in patients with a DCM-like presentation, particularly in advanced disease stages. This phenotypic overlap blurs the diagnostic boundary between ARVC and DCM and highlights the diagnostic complexity of *DSG2*-related disease. Moreover, heterozygous *DSG2* variants have been identified in individuals with SCD, even in the absence of structural disease, while others are discovered incidentally during screening in asymptomatic individuals.

An additional layer of complexity arises from emerging data suggesting inflammatory mechanisms in *DSG2*-related ACM, particularly in homozygous carriers, a finding also observed in our Padua cohort in the homozygous *DSG2* c.1672C>T p.(Gln558*) carrier. Although rare, clinical cases and animal models have demonstrated myocarditis-like presentations that may precede structural remodeling and fibrosis, suggesting a potential immunomodulatory component to disease progression [55, 56, 57].

In our Padua cohort, most *DSG2* carriers met the revised Task Force criteria (2010TFC), with classical features such as precordial T-wave inversion, RV dilation, and dysfunction [14]. Although mean LVEF was preserved, a significant proportion of patients exhibited a biventricular form according to the Padua criteria, primarily due to frequent LV fibrofatty replacement [15]. While prior studies have consistently reported more pronounced LV involvement and a higher risk of transplantation or heart failure-related death in *DSG2* carriers compared to other ACM-related genes (e.g., *PKP2*, *DSC2*), our cohort displayed a relatively low heart transplantation rate (4%)

and preserved systolic function [33, 49, 52, 58]. Nonetheless, arrhythmic events were frequent, with a high prevalence of MVA, consistent with previous literature [12, 59]. This pattern likely reflects earlier detection via family screening and application of the Padua criteria, which identify LV disease prior to pump failure, and longitudinal follow-up, all of which may delay progression to end-stage heart failure.

Despite preserved ejection fraction in many patients, the arrhythmic burden remains high, highlighting the necessity for rigorous and individualized risk stratification. In this context, our study supports the clinical utility of long-term follow-up and the ARVC Risk Calculator as a predictive tool. The strong correlation between predicted and observed arrhythmic outcomes over a five-year horizon validates its application in guiding clinical decisions, ICD implantation strategies, and broader preventive interventions in *DSG2* variant carriers. Taken together, these findings affirm that *DSG2*-related ACM represents a clinically heterogeneous entity, capable of mimicking both ACM and DCM, with potential inflammatory features in select cases.

Importantly, this work elucidates under-recognized mechanisms of *DSG2*-ACM inheritance. While autosomal dominant transmission has long been considered the prevailing model, our findings support a broader genetic framework that includes biallelic inheritance patterns, namely homozygous and compound heterozygous variants, which are consistently linked to earlier disease onset, more extensive ventricular involvement, and a higher arrhythmic burden. These biallelic cases frequently present in adolescence or early adulthood resulting in completely penetrant ACM phenotypes with rapid clinical progression, severe arrhythmias and, in some instances, heart failure necessitating transplantation [27, 30, 33, 45]. In contrast, heterozygous relatives of affected probands often remain asymptomatic or present with only mild or subclinical features, may be influenced by environmental factors, and inter-individual genetic background leading to a variable penetrance of *DSG2* variants [30].

In addition to biallelic inheritance, our study highlights the pathogenic relevance of digenic inheritance where *DSG2* variants co-occur with variants in other desmosomal genes such as *PKP2* or *DSP* adding another layer of complexity to the genetic landscape of ACM. These multilocus mechanisms underscore the limitations of conventional single-gene testing and emphasize the need for comprehensive molecular diagnostics, including CNV analysis and broad gene panel screening, particularly in cases presenting with atypical or severe phenotypes.

Together, these findings reinforce the importance of considering non-dominant and multilocus inheritance in *DSG2*-related ACM and support the implementation of individualized risk assessment strategies, family-based screening, and long-term clinical surveillance.

The widespread availability of genetic testing has greatly enhanced the ability to identify genotype-positive individuals at risk of life-threatening arrhythmias and to implement timely prophylactic interventions. However, it has also exposed key challenges in ACM, namely incomplete penetrance and variable expressivity, even among carriers of the same pathogenic *DSG2* variant. These phenomena are particularly evident in familial context.

This intra-familial variability in phenotypic expression points to the influence of genetic modifiers and synergistic interactions with additional variants in desmosomal or other cardiomyopathy-related genes, collectively referred to as genetic background [60, 61, 62, 63]. Adding to this complexity, our study also highlights the role of CNVs in *DSG2*, which may act as primary pathogenic events in cases lacking identifiable point mutations.

Low-frequency variants in desmosomal genes may function as modifiers by subtly impairing desmosomal integrity, which, when combined with a primary *DSG2* genetic variant, may unmask or intensify disease expression. In some cases, a "second hit" either as compound heterozygosity within *DSG2* or digenic heterozygosity involving another desmosomal gene such as *PKP2* or *DSP* may be required for overt clinical manifestation. Individuals harboring multiple desmosomal variants often demonstrate higher penetrance and more severe phenotypes, supporting a multilocus inheritance model [31, 36, 64, 65, 66].

Our study enriches this multifaceted field by identifying a 42% penetrance among *DSG2*-positive carriers, a figure that lies just below the 52% previously documented in other genotype-defined cohorts. [44, 67]. The observed penetrance in our cohort likely reflects the combined influence of heterozygous low-penetrance alleles and a subset of fully penetrant homozygous cases. This observation underscores the variable expressivity and zygosity-dependent risk in *DSG2*-related ACM. This observation likely reflects the combined influence of zygosity, modifier alleles, and environmental factors, and underscores the necessity of systematic family screening and longitudinal monitoring of asymptomatic carriers. Ultimately, integrating comprehensive genotypic profiling with detailed phenotypic assessment is critical for refining risk stratification and guiding personalized management in *DSG2*-related ACM.

Within a structure–function framework, the structural domains of DSG2 are clearly established as critical for maintaining cardiac desmosome integrity [7]. The N-terminal pro-peptide of DSG2 contains a conserved furin recognition sequence (Arg-Gln-Lys-Arg; residues 46–49), essential for

pro-domain cleavage and subsequent desmosomal integration. Aminoacidic variations within this motif, such as p.(Arg46Trp), p.(Arg46Gln), p.(Arg49His), and p.(Lys48Asn), have been associated with aberrant post-translational processing [27, 68] (Figure 1B). Although these variants do not impede DSG2 transport to the plasma membrane, they may alter binding dynamics, as seen with p.(Arg46Gln), which exhibits enhanced binding capacity and potential mutant dimerization, potentially disrupting wild-type interactions [7, 64].

Such alterations can compromise calcium-mediated trans-interactions, weakening desmosomal adhesion despite increased binding affinity.

Post-translational modifications, including N-glycosylation at five sites (N112, N182, N309, N462, N514) and O-mannosylation at three sites (T480, T482, T484), along with proper trafficking through the endoplasmic reticulum and Golgi apparatus, are vital for DSG2 maturation [69, 70]. In this context, certain variants located in specific regions may affect the transport or maturation of DSG2 protein, thereby influencing its functionality and/or localization.

The 117kDa mature DSG2 protein comprises four extracellular cadherin domains (EC1–EC4) with essential calcium-binding sites (Figure 1A) necessary for establishing homophilic-heterophilic intercellular associations and forming Ca^{2+}-dependent rod-like structures. Variants affecting these residues, such as E230, D243, N266, E283, D297, D419, and D519, can impair calcium binding, reducing adhesive function. In our cohort, compound heterozygous variants *DSG2* c.689A>G p.(Glu230Gly) and c.890A>G p.(Asp297Gly) were identified *in trans* configuration in a female proband, underscoring the pathogenic potential of alterations in calcium-binding sites. We assessed the predicted pathogenicity of these missense variants based on amino acid substitutions (Figure 4A) and evaluated their impact on protein stability by calculating the ΔΔG values (Figure 4B).

Truncating variants leading to premature stop codons can result in DSG2 proteins lacking critical cytoplasmic components, including the PKP2-binding site located in the ICS region (Figure 1C). While these truncated proteins may retain the transmembrane domain, they disrupt essential protein interactions, compromising desmosome stability and cytoskeletal anchoring, thereby predisposing to conduction abnormalities [29]. Functional studies of ICS variants suggest altered binding affinity as a key pathological mechanism [64, 71, 72]. Notably, DSG2 possesses the longest repeat unit domains (RUDs) among its isoforms, which enhance adhesion by preventing

internalization and stabilizing membrane presence. Truncating mutations affecting the RUD region have been linked to increased DSG2 internalization and compromised cell adhesion [72].

Classification of all variants in the literature, gnomAD, and ClinVar does not include evidence that requires manual curation from the literature segregation (PP1), functional data (PS3/BS3), de novo inheritance (PS2/PM6). The retrospective design of this study resulted in missing clinical data for some older cases, particularly incomplete imaging datasets for CMR, which was not systematically performed during the early diagnostic period. This incomplete data capture represents a limitation and may slightly reduce the precision of certain clinical correlations.

Conclusions

This study provides a comprehensive and integrative evaluation of *DSG2* variants in ACM combining systematic literature review, curated population-scale databases, and a deeply phenotyped Italian validation cohort. By mapping variants across structural domains and applying ACMG criteria, we refined the interpretation of *DSG2* pathogenicity and identified domain-specific mutational hotspots, notably in calcium-binding extracellular domains, the furin cleavage site, and the PKP2-binding region.

Importantly, our findings refine the current understanding of *DSG2*-related ACM inheritance. We confirm and contextualize the contribution of biallelic and digenic mechanisms, as well as pathogenicity of CNVs, which in our cohort are associated with earlier disease onset, greater arrhythmic burden, and more extensive ventricular involvement. We also illustrate the clinical value of identifying potentially high-impact missense variants ("hot-VUS") to aid reclassification and risk assessment. Together, these observations strengthen the evidence base for genotype-informed evaluation and follow-up of individuals and families carrying *DSG2* variants.

Through structure-function modeling and ΔΔG protein stability predictions, this study links variant location and molecular disruption with clinical phenotype. These insights strengthen the foundation for genotype-informed risk stratification and illustrate the critical role of *DSG2* in desmosomal stability and cardiac conduction.

Finally, by estimating variant penetrance at 42% and validating the utility of the ARVC Risk Calculator in a real-world setting, our work underscores the need for long-term clinical surveillance and systematic family screening of *DSG2* mutation carriers. These findings advocate for a shift toward comprehensive, multilocus, and integrative genomic frameworks in ACM diagnostics, ultimately supporting the future of precision medicine in inherited cardiomyopathies.

Data availability statement

The full-length DSG2 protein structure was obtained from the AlphaFold Protein Structure Database (UniProt accession: Q14126; model AF-Q14126-F1, version v4). *DSG2* genetic variants were systematically collected from publicly available databases, including gnomAD v4.1.0 (https://gnomad.broadinstitute.org) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). No newly generated sequencing data were deposited in public repositories. Clinical data, including ECG, echocardiography, and CMR evaluations were collected according to current ESC 2023 Guidelines.

Due to ethical and privacy constraints related to human genetic and clinical data, the raw data generated and analyzed in this study are not publicly available. Access to the raw data may be granted upon reasonable request and subject to approval by the corresponding author, in accordance with institutional policies and applicable regulations. Requests should be addressed to Kalliopi Pilichou (email: kalliopi.pilichou@unipd.it). A response to data access requests will be provided within 15 working day**s** of receipt. Approved access will be subject to a data use agreement defining conditions of use, including restrictions on data sharing, requirements for appropriate data protection, and limitation of use to non-commercial research purposes.

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Figure caption

Figure 1. Schematic representation of the Desmoglein-2 (DSG2) protein domains highlighting identified variants in literature and in Padua cohort.

(A) Missense variants are indicated by circles, while radical variants are shown as squares. Variants reported in the literature are depicted in violet, whereas those identified in the Padua cohort are shown in red. (B-C) Multiple sequence alignment of DSG2 sequences obtained using Clustal, highlighting conserved amino acid regions across different proteins. Conserved residues are marked by specific symbols (e.g., '*', ':', '.'), indicating the degree of similarity between aligned positions: (B) Furin recognition motif, (C) and PKP2 binding site. Pro-peptide region (Pro), extracellular cadherin homology domains (ECs 1-4), extracellular anchor (EA), single transmembrane domain (TM), intracellular anchor (IA), cadherin-specific intracellular segment (ICS), proline-rich linker (LD), six repeat unit domains (RUDs), and a glycine-rich terminal domain (TD). Red boxes highlight most conserved residues.

Figure 2. **Frequency and distribution of *DSG2* variants across protein domains classified by clinical significance.**

DSG2 variants as reported by (A) literature, (B) the gnomAD, and (C) ClinVar databases, and (D) Padua cohort and classified by clinical significance. The colors represent different variant classifications: green B (Benign), blue LB (Likely Benign), yellow VUS (Variant of Uncertain Significance), orange LP (Likely Pathogenic), and red P (Pathogenic).

Figure 3. Representative cases with *DSG2* variants.

A-D) SCD case of a *DSG2* c.1652-1C>T male carrier who died suddenly at the age of 25. A) Gross view of the heart in cross section with biventricular involvement (RV, right ventricle; arrow pointing to the left ventricular -LV- postero-basal wall). B-C) Postero-lateral left (B) and right (C) ventricular free wall histology with evidence of subepicardial and transmural mostly fibrous replacement with preserved wall thickness (Heidenhain's Azan trichrome stain; panoramic view); in D, 'cardiomyopathic' changes of myocytes are shown (scale bar 100 mm). E-H) Explanted heart of a 65-year-old woman with c.797A>G variant. E) Cross-section gross view of the heart with transmural tissue replacement of the right ventricular (RV) free wall and subepicardial, focally transmural, of the left ventricular (LV) free wall (arrow). Postero-lateral left (F) and right (G) ventricular histology showing transmural fibro-fatty replacement of the myocardium coupled with wall thinning (Heidenhain's Azan trichrome stain; panoramic view); in H), high magnification of ventricular myocardium showing perinuclear haloes (white arrows) and dystrophic calcification of subendocardial ventricular myocardium (black arrow, scale bar 100 mm).

Figure 4. Prediction of calcium-binding residues of DSG2 protein.

(A) Heat map of all possible missense variants in calcium binding residues of DSG2 protein. Black rectangular identifies amino acid present in the reference UniProt protein sequence, based on hg38. (B) Impact of E230G and D297G on DSG2 stability PDB (AF-Q14126-F1-model_v4) identified in Padua cohort. The DynaMut server was used to calculate the predicted change in stability (in kcal/mol) for each variant.

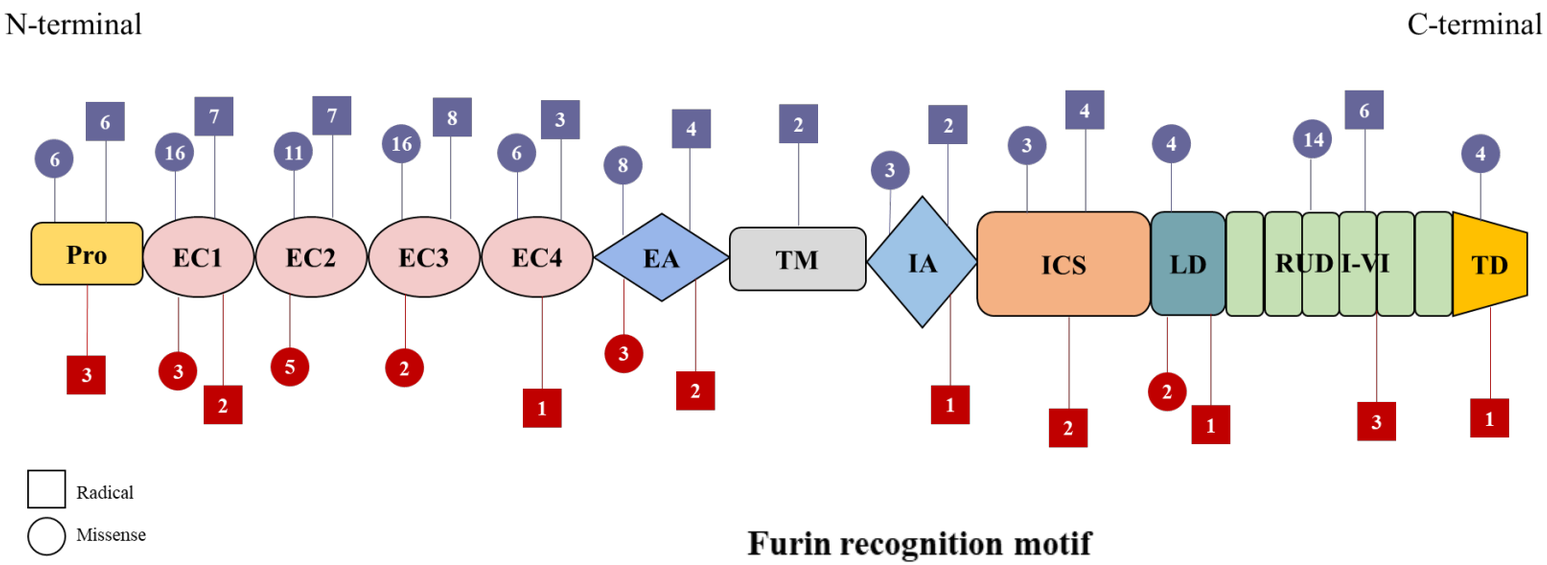
Figure 5. Pedigrees of the four available families carrying compound/double heterozygous variants.

Each pedigree illustrates the inheritance pattern and segregation of the identified variants within the respective family. N.A. not available; * obligate carrier; - genetically negative. Square: male. Circle: Female. Black square and circle: affected. White square and circle: healthy.

ED SUM:
Pinci et al. examine the link between genetic variants in the DSG2 gene and arrhythmogenic cardiomyopathy by integrating population, literature, and clinical data. They show that disease risk depends on variant type, location, and multilocus interactions, refining genetic diagnosis and risk assessment.

Peer review information:
Communications Medicine thanks Roddy Walsh, Jasper J. van der Smagt and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. A peer review file is available.

A.



B.

Furin recognition motif

```
tr|A0A5F5XXT4|A0A5F5XXT4_FELCA  SAEGAALLLLICFNFGNGLHLEALNTRNENNLLPKHTHLNRQKRAWITAP
tr|A0A8I3RY38|A0A8I3RY38_CANLF  SAEGAALLVLICFYFGNGLHLEVLNARNGNTLLPKHRHLVRQKRAWITAP
sp|Q14126|DSG2_HUMAN            GRAYALLLLLICFNVGSGLHLQVLSTRNENKLLPKHPHLVRQKRAWITAP
sp|O55111|DSG2_MOUSE            LLLLVQLLAVVCLDFGNGLHLEVFSPRNEGKPFPKHTHLVRQKRAWITAP
tr|A0A8I6AG90|A0A8I6AG90_RAT    DTPTPTRSPSNSITPWDLTIHFGNLTRGEDRLFTKRTHLVRQKRAWITAP
tr|A0A8V0XGN8|A0A8V0XGN8_CHICK  SAAARLLALLICLSCGNGLHWKIHNRSGRSGTLFHPNSLVRQKREWTVPP
sp|H2EQR6|DSG21_DANRE           RRISPVVAFLLCFGLSHFFEAEAR--------LQHSVALHRQKREWIVPP
                                          .:                  : :   * **** * ..*
```

C.

PKP2 binding site

```
tr|A0A5F5XXT4|A0A5F5XXT4_FELCA  LVYSQEDTASLHGSIGCCSFIEGELDDHFLDDLGLKFRTLAEVCLGQKID
tr|A0A8I3RY38|A0A8I3RY38_CANLF  LVYSQEDTASLHGSIGCCSFIEGELDDGFLDDLGFKFRTLAEICLGQKID
sp|Q14126|DSG2_HUMAN            LVYSQEETESLNASIGCCSFIEGELDDRFLDDLGLKFKTLAEVCLGQKID
sp|O55111|DSG2_MOUSE            LVYSQEDTASLRGSVGCCSFIEGELDDLFLDDLGLKFKTLAEVCLGRKID
tr|A0A8I6AG90|A0A8I6AG90_RAT    LVYSQEDTDSLRGSVGCCSFIEGELDDLFLDDLGPKFKTLAEVCLGRKID
tr|A0A8V0XGN8|A0A8V0XGN8_CHICK  LVYSQGESGSPHGSIGCCSFIEGDLDDHFLDDLGDKFKTLAEICIGRRID
sp|H2EQR6|DSG21_DANRE           MVYDYEGKGSPVGSVGCCSLLEDQNDLEFLNDLGPKFTTLADICGGKKTE
                                :**.   . *  .* ****:.*.: *  **:*** ** ***::* *:: :
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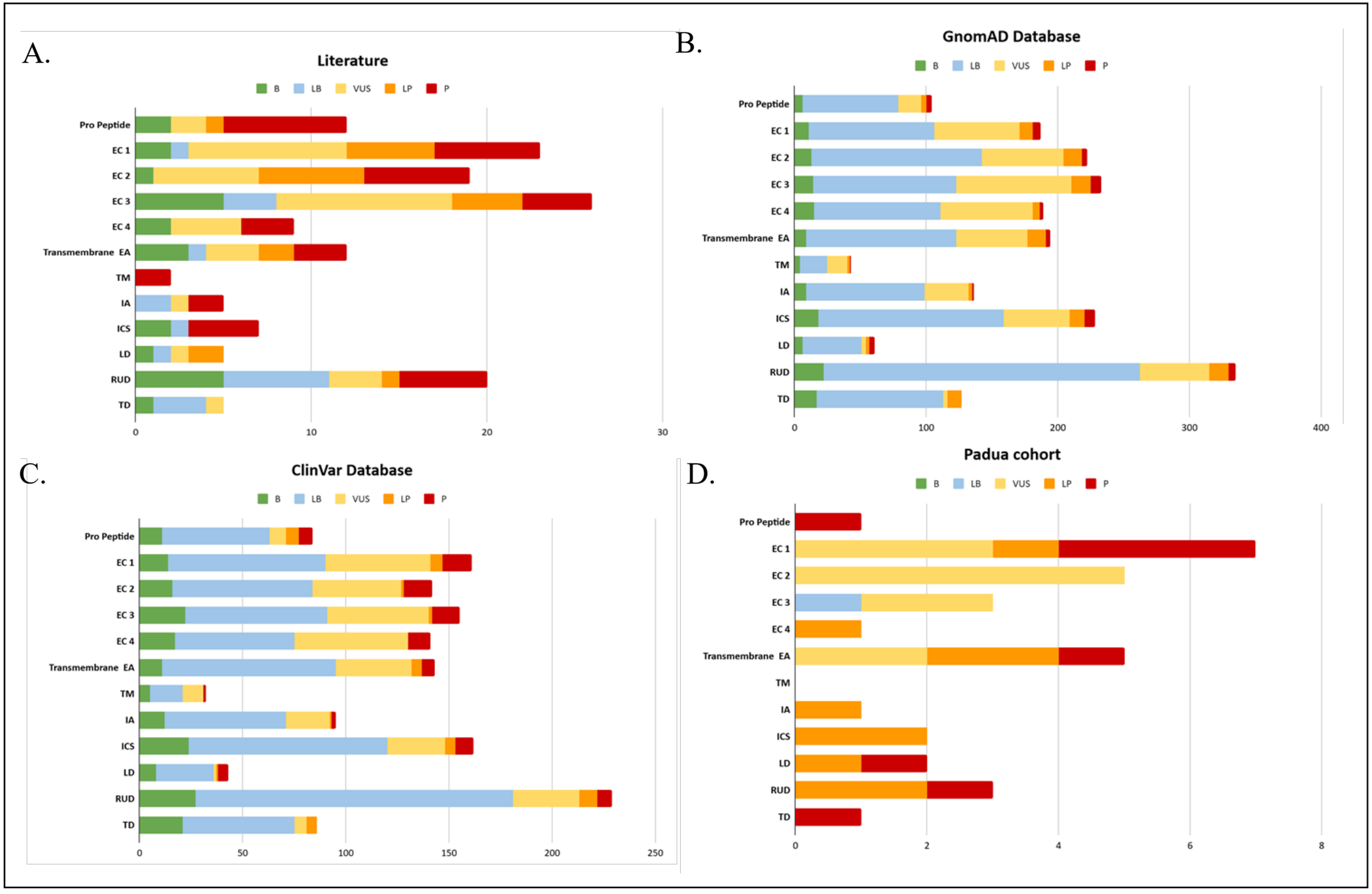
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LP
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EC 3
EC 4
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LD
RUD
TD
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10
20
30
B.
GnomAD Database
B
LB
VUS
LP
P
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EC 2
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100
200
300
400
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EC 4
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200
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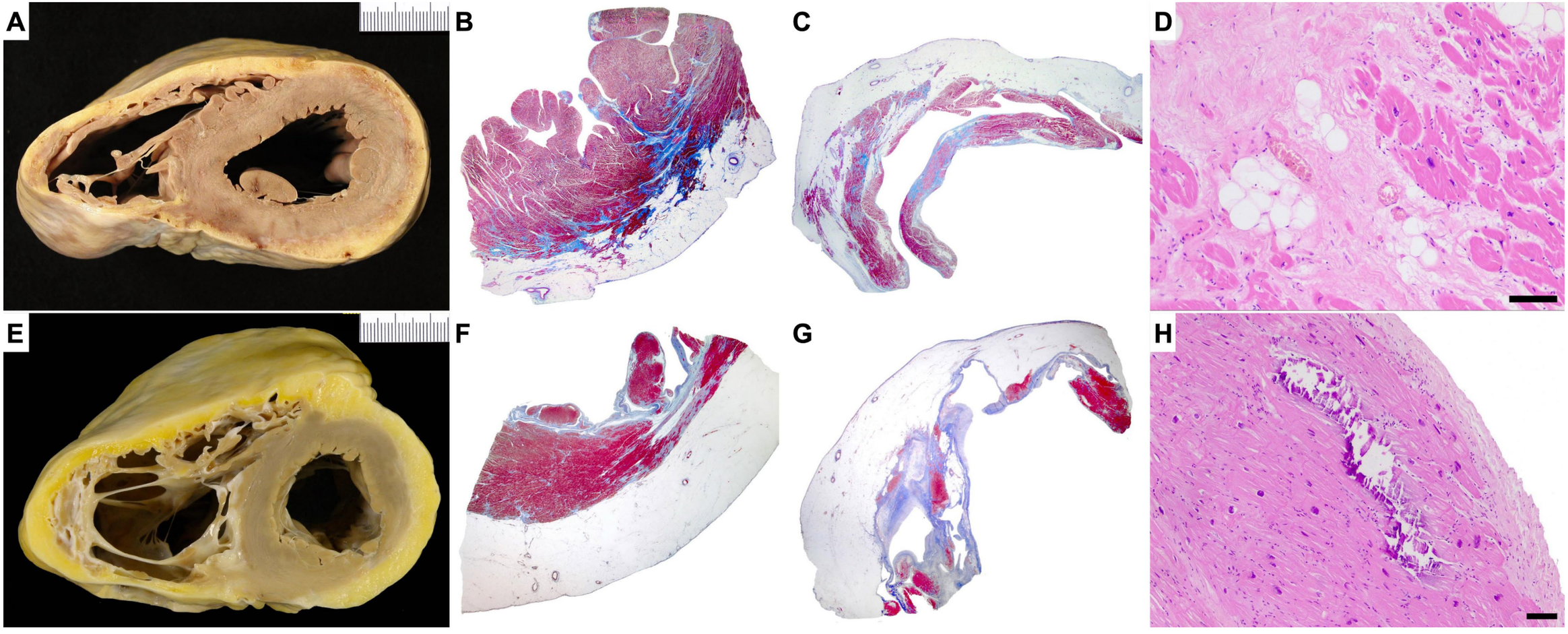
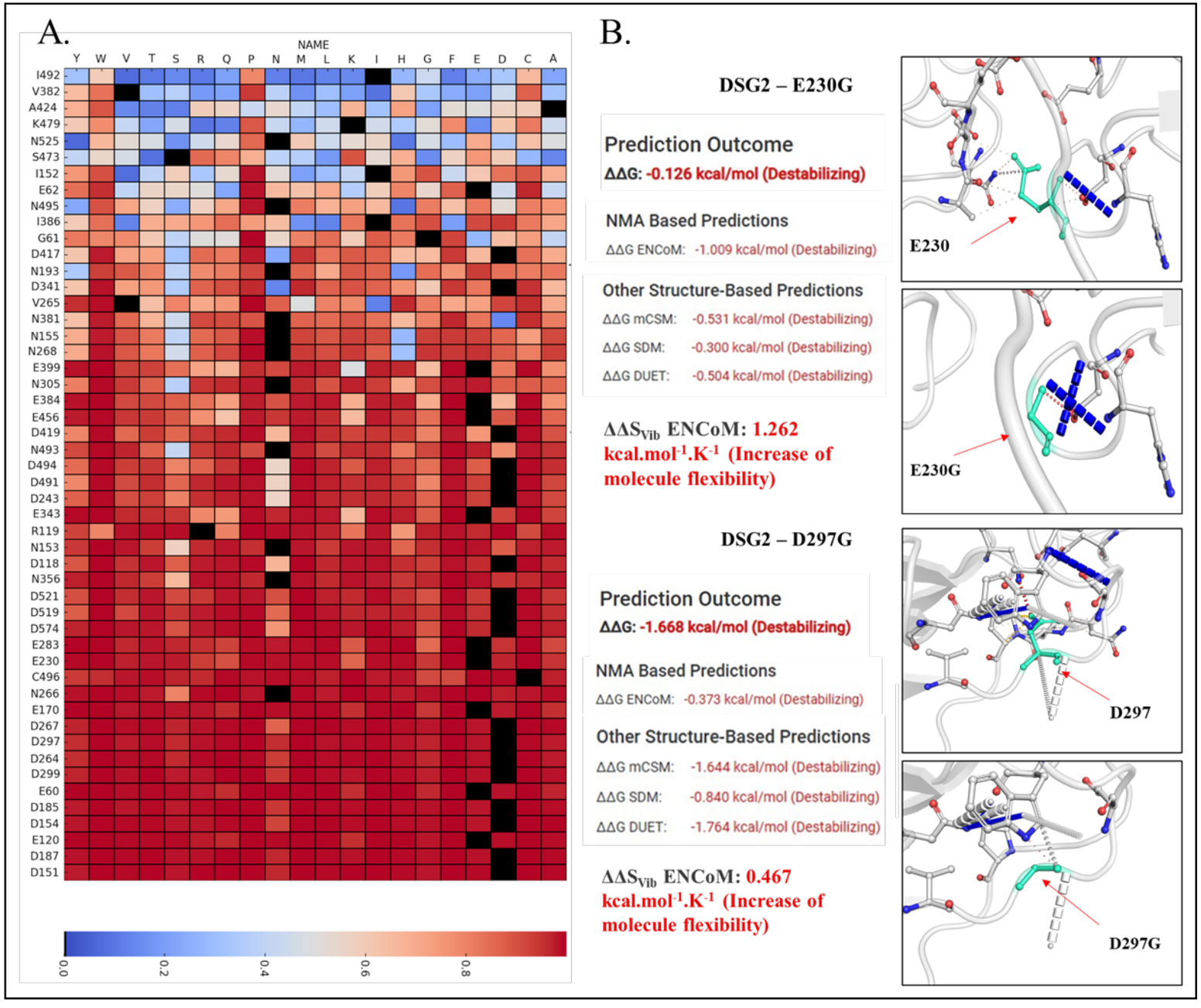

A
B
C
D
E
F
G
H


A.
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I386
G61
D417
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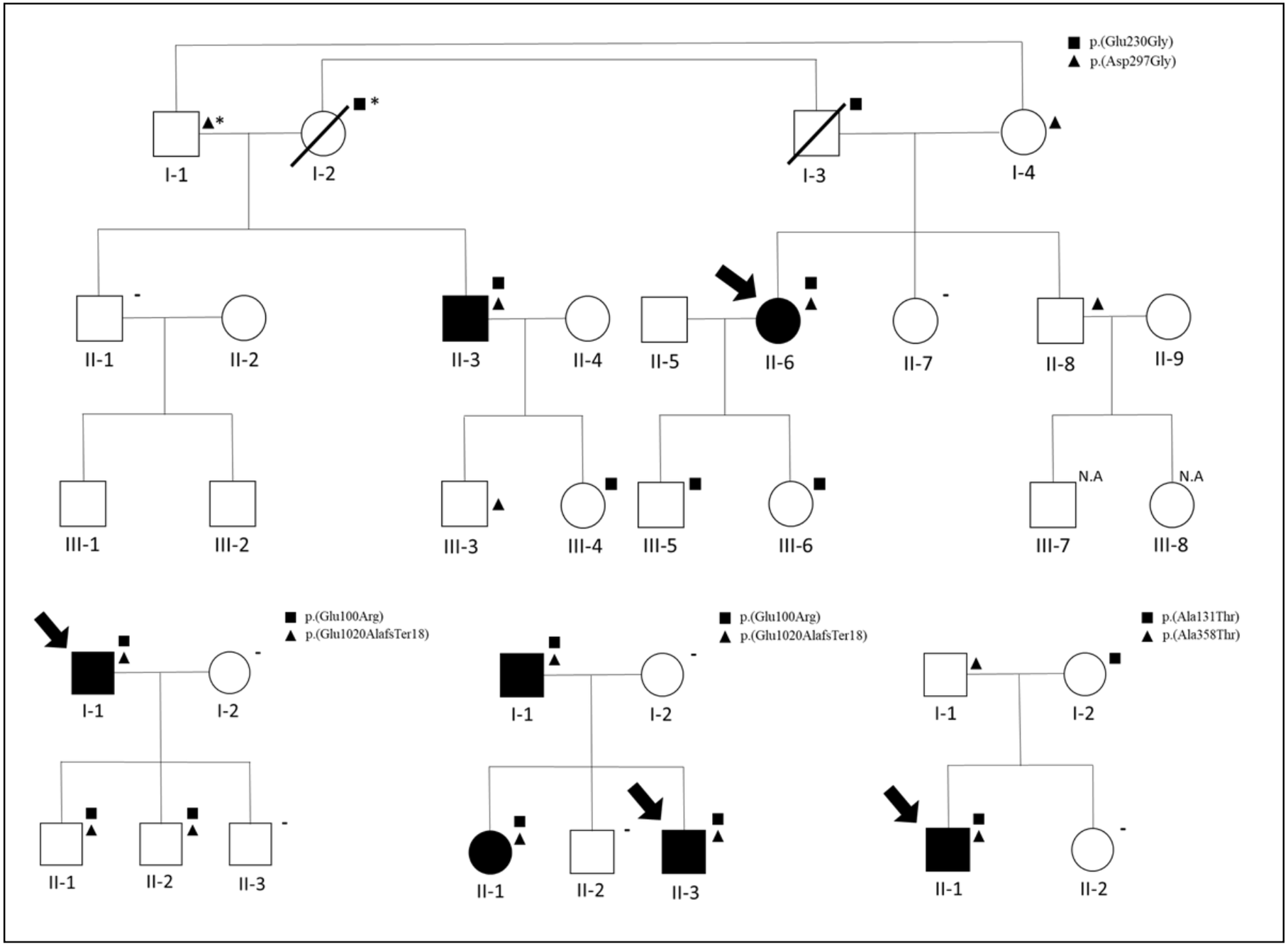
D341
V265
N381
N155
N268
E399
N305
E384
E456
D419
N493
D494
D491
D243
E343
R119
N153
D118
N356
D521
D519
D574
E283
E230
C496
N266
E170
D267
D297
D264
D299
E60
D185
D154
E120
D187
D151
0.0
0.2
0.4
0.6
0.8
B.
DSG2 – E230G
Prediction Outcome
ΔΔG: -0.126 kcal/mol (Destabilizing)
NMA Based Predictions
ΔΔG ENCoM: -1.009 kcal/mol (Destabilizing)
Other Structure-Based Predictions
ΔΔG mCSM: -0.531 kcal/mol (Destabilizing)
ΔΔG SDM: -0.300 kcal/mol (Destabilizing)
ΔΔG DUET: -0.504 kcal/mol (Destabilizing)
ΔΔS_Vib ENCoM: 1.262 kcal.mol-1.K-1 (Increase of molecule flexibility)
E230
E230G
DSG2 – D297G
Prediction Outcome
ΔΔG: -1.668 kcal/mol (Destabilizing)
NMA Based Predictions
ΔΔG ENCoM: -0.373 kcal/mol (Destabilizing)
Other Structure-Based Predictions
ΔΔG mCSM: -1.644 kcal/mol (Destabilizing)
ΔΔG SDM: -0.840 kcal/mol (Destabilizing)
ΔΔG DUET: -1.764 kcal/mol (Destabilizing)
ΔΔS_Vib ENCoM: 0.467 kcal.mol-1.K-1 (Increase of molecule flexibility)
D297
D297G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