SIMPOSIO ANNUALE DEL CENTRO
DI RIFERIMENTO PER LA SINDROME
DI MARFAN E PATOLOGIE CORRELATE
FOCUS SULLA SINDROME
DI EHLERS-DANLOS VASCOLARE

17 maggio 2025, ore 9:30-17:00
Aula Anfiteatro Giubileo 2000 - Policlinico Tor Vergata
Viale Oxford 81, 00133 - Roma

IL PRESENTE:
PRINCIPI DI PERSONALIZZAZIONE DELLE CURE DELLA PERSONA CON ARTERIOPATIA/AORTOPATIA EREDITARIA

La diagnostica di laboratorio oggi in cardiogenetica: come e perché

Marco Castori; MD, PhD
UOC Genetica Medica
Fondazione IRCCS-Casa Sollievo della Sofferenza

con il patrocinio di

SUSPICION

DIAGNOSIS

Laboratory report

PROGNOSIS

PREVENTION

FAMILY PLANNING

TREATMENT

Major determinants to the clinical utility of a genetics report:

- ✓ Reliability
- ✓ Understandability
- ✓ Rapidity

CLINICS

Functional data

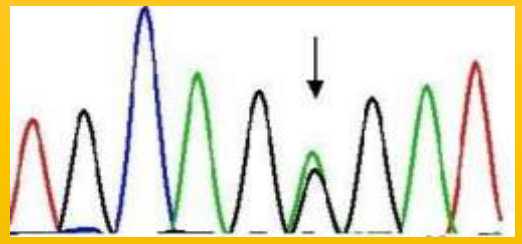
Phenotypic data

Family/segregation data

CLINICAL CONTEXT

Laboratory report

INDIVIDUAL'S DNA



Sequencing data

HUMAN VARIOME

Global information

*«[…] The identified genotype, which falls in a **gene** and belongs to a **type** previously associated with the clinical suspicion, is interpreted as the 'cause' of the individual's disease because enough data support **causation** […]»*

LABORATORY

DATABASES

PATHOGENIC VARIANTS

LIKELY PATHOGENIC VARIANTS

VARIANTS OF UNCERTAIN SIGNIFICANCE

LIKELY BENIGN VARIANTS

BENIGN VARIANTS

16 criteria of pathogenicity

PVS1	*null* allele (stopgain, frameshift, D/A splice variant) in a gene with LoF
PS1	different nt change but same aa change known as deleterious
PS2	*de novo* allele with parental status origin
PS3	functional documentation of pathogenicity
PS4	allele statistically more common in cases compared to controls
PM1	variant falling in a mutational hot spot and/or critical domain
PM2	variant absent or rare in population databases
PM3	variant in trans with a known deleterious variant (AR genotypes)
PM4	in-frame insertion/deletion falling in non-repetitive regions
PM5	different aa change at the same codon known as deleterious
PM6	*de novo* allele without documented parental origin
PP1	variant co-segregating with the disease in other family members
PP2	missense change in a gene with low rate of benign missense changes
PP3	missense change predicted deleterious *in silico*
PP4	phenotype specific for the involved gene
PP5	variant reported as deleterious in public databases

12 criteria of benignity

BA1	Allele with a VAF >0.05 in population databases
BS1	allele with a VAF too high for the presumed disease frequency
BS2	allele previously observed in healthy individuals
BS3	functional documentation of a neutral effect
BS4	lack of segregation with the disease within the family
BP1	missense change in a gene with high rate of benign missense changes
BP2	observed in combination of a deleterious genotype at the same locus
BP3	in-frame insertion/deletion in a repetitive region
BP4	missense change predicted neutral/non-deleterious *in silico*
BP5	observed in combination with an alternative genetic cause
BP6	variant reported as neutral/non-deleterious in public databases
BP7	synonymous change predicted not affecting splicing *in silico*

POSITIVE RESULTS

PLP hemizygous variant in a suspicion-related XLR gene
PLP heterozygous variant in a suspicion-related dominant gene
Biallelic PLP variants in a suspicion-related AR gene

INCOMPLETE RESULTS

PLP heterozygous variant in a suspicion-related AR gene
Double PLP heterozygous variants in a suspicion-related AR gene

SECONDARY RESULTS

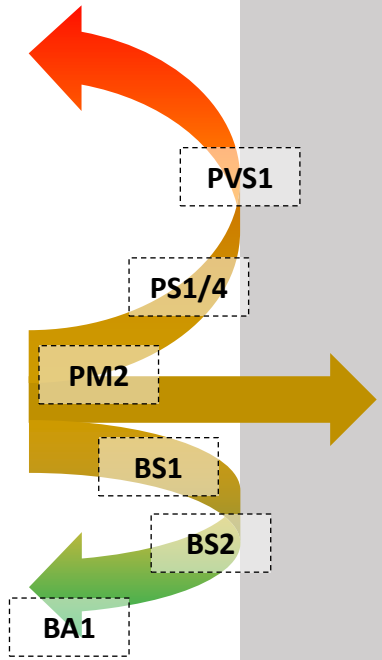
PLP variant(s) in a suspicion-unrelated but actionable gene

INCONSISTENT RESULTS

One or more VUS in a suspicion-related gene

NEGATIVE RESULTS

Variants not causative in suspicion-related genes
Variants not causative in actionable genes



LABORATORY-CLINICS INTERACTIONS

PS2/PM6	*de novo* variant
PS3/PVS1s	*in vitro* functional effect
PM3	in *trans* with another PLP variant
PP1	co-segregation with the disease
PP4	specificity of the phenotype

LABORATORY

VUS with hypothetical effect in a suspicion-related gene
VUS+/variants of interest (VOI)
e.g. private missense/intronic/synonym with a presumed effect in silico/databases e.g. private in-frame indels

PVS1 (downgraded), PM1, PM4, PM5, PP2, PP3, PP5

Rare variants without enough criteria for the PLP or BLB status

BP1, BP3, BP4, BP6, BP7

VUS without a hypothetical effect in a suspicion-related gene
e.g. private missense/intronic/synonym predicted benign/neutral in silico/databases

BS2	found in healthy individuals
BS3/BP7	*in vitro* functional effect
BS4	lack of segregation
BP2	in combination of other PLP variants
BS5	in combination with another cause

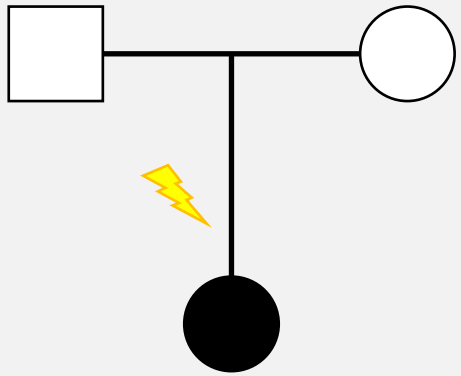
CRITERIA RELATED TO PHENOTYPE SPECIFICITY

PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history
Note: ***Confirmation of paternity only is insufficient***. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity.

PM6 Assumed de novo, but without confirmation of paternity and maternity

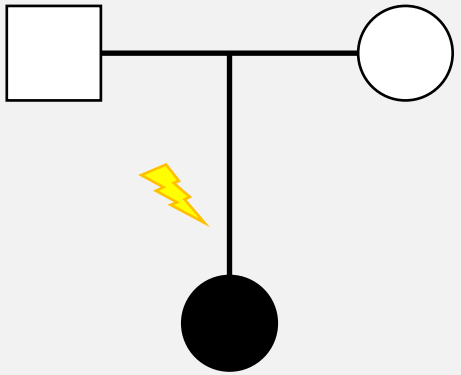
*[…] The phenotype in the patient matches the gene's disease association with **reasonable specificity**. For example, **this argument is strong** for a patient with a de novo variant in the NIPBL gene who has distinctive facial features, hirsutism, and upper-limb defects (i.e., Cornelia de Lange syndrome), **whereas it would be weaker** for a de novo variant found by exome sequencing in a child with nonspecific features such as developmental delay […].*

Aspecific/generic phenotype
High locus heterogeneity
(e.g. **TAAD**)



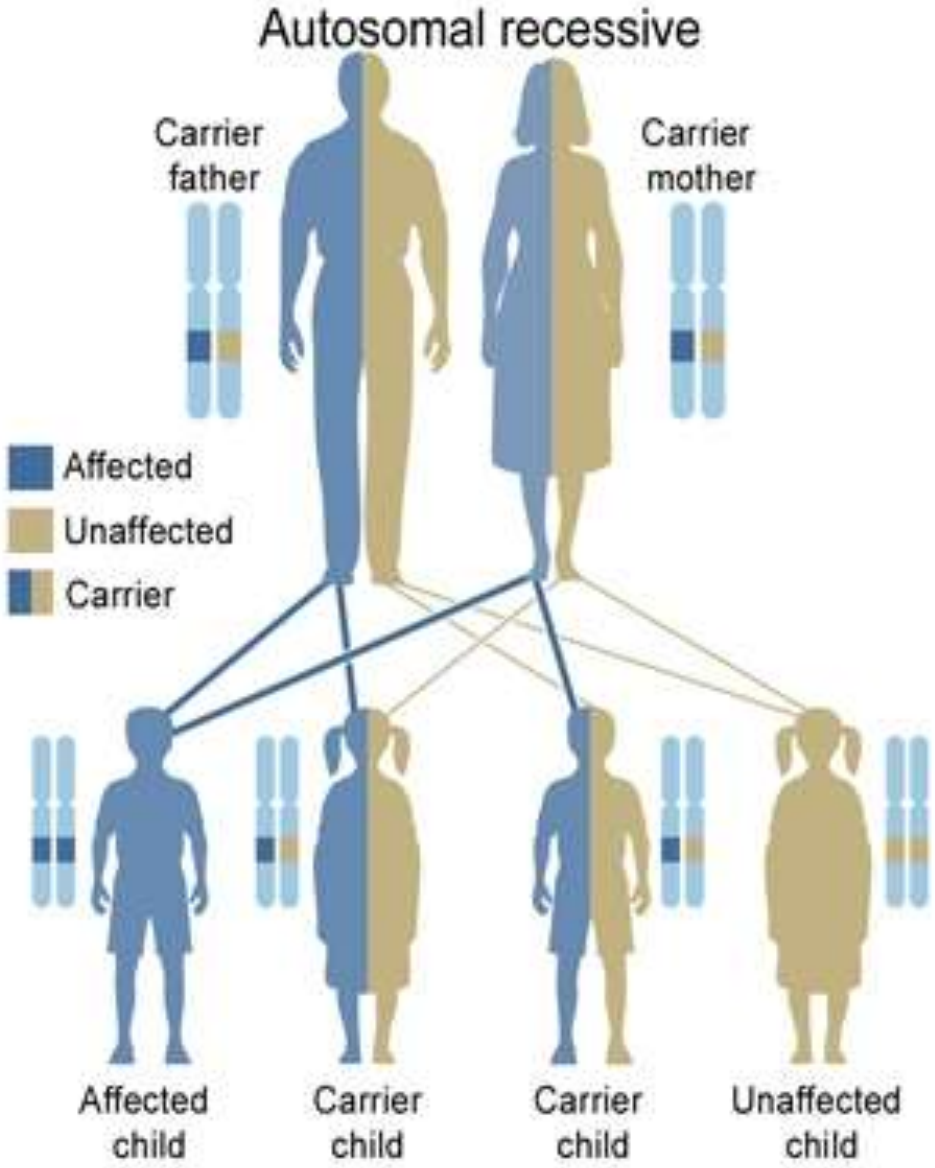
PM6_Supporting
PS2_Moderate

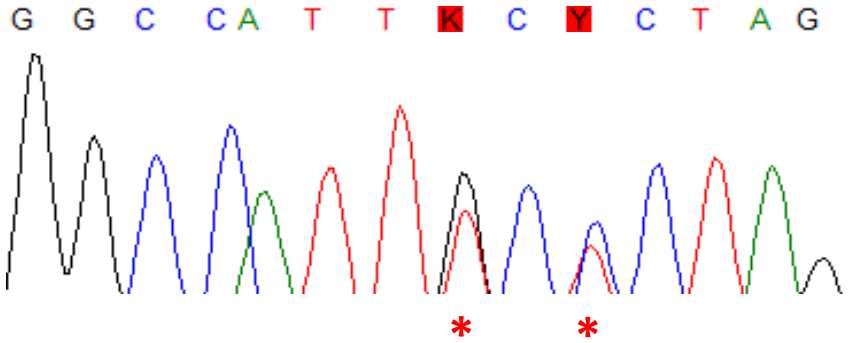
Specific phenotype
Very low locus heterogeneity
(e.g. **Marfan syndrome – Ghent criteria met**)



PM6_Moderate
PS2_Strong

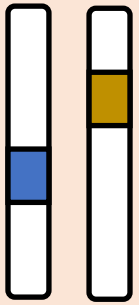
THE NEED OF PHASE STUDY IN AUTOSOMAL RECESSIVE DISEASES





In the absence of segregation data from first-degree relatives…

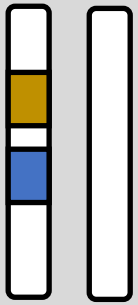
HYPOTHESIS 1



The variants are in TRANS

The genotype is CAUSATIVE

HYPOTHESIS 2

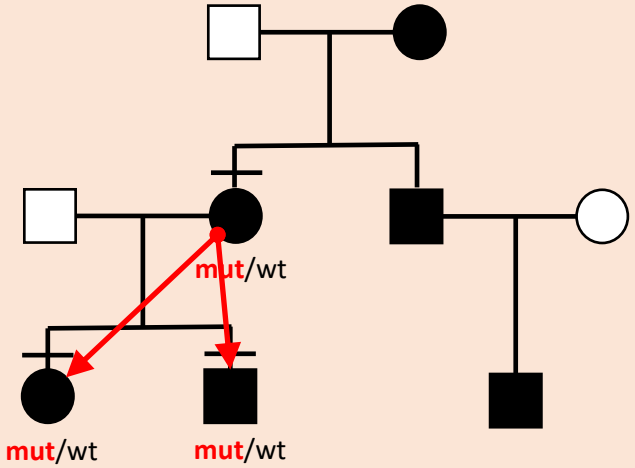


The variants are in CIS

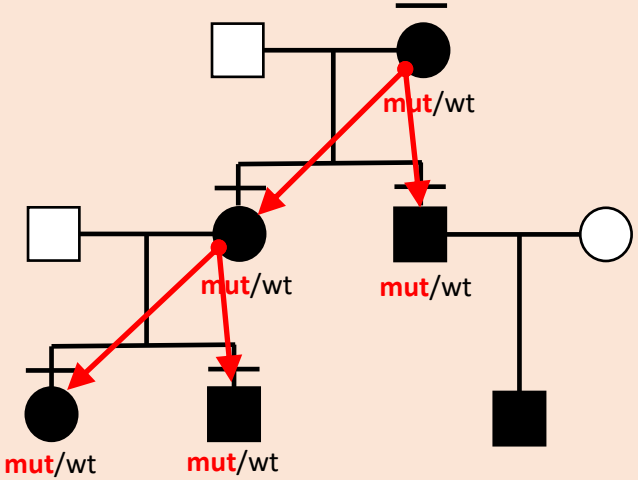
The genotype is NOT CAUSATIVE

CRITERIA RELATED TO SEGREGATION DATA

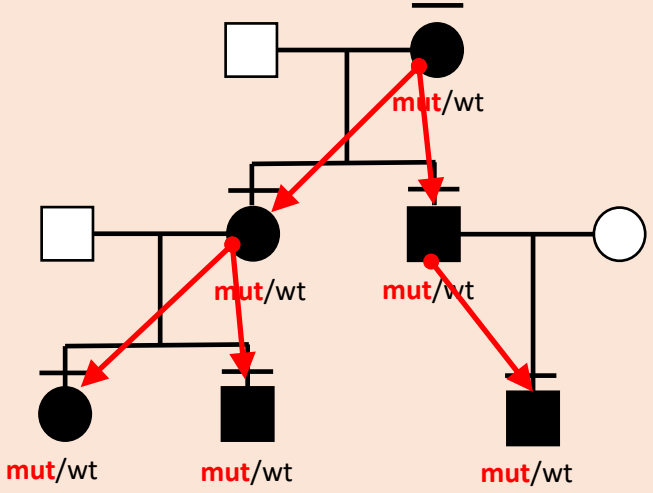
AD: PP1_Supporting



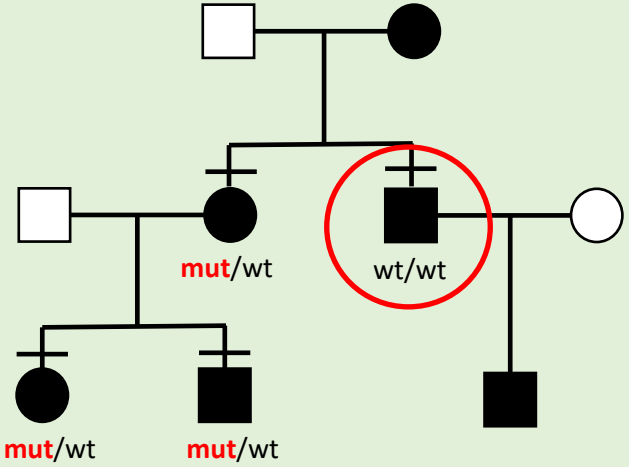
AD: PP1_Moderate



AD: PP1_Strong

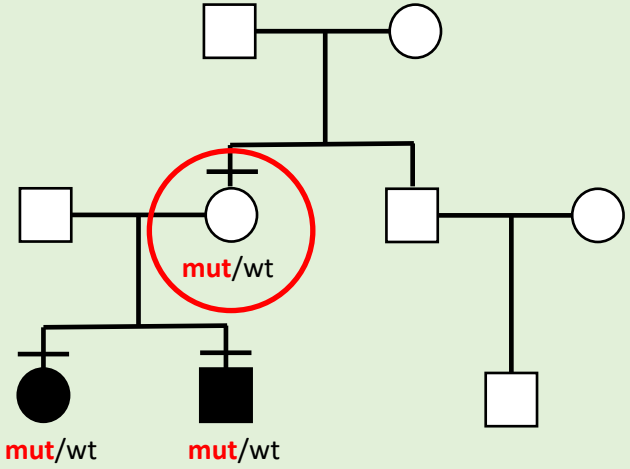


AD: BS4_Strong

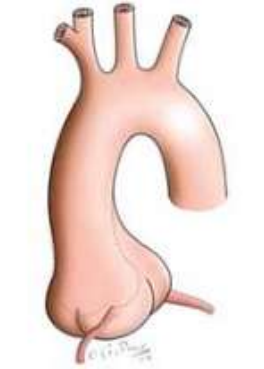


AD: BS2_Strong

If complete penetrance in the pediatric age



CRITERIA RELATED TO PHENOTYPE SPECIFICITY



Aortic root diameter Z-score (adult): >2 SD

Non-specific, high locus heterogeneity

→No PP4

Aortic root diameter Z-score: >2 SD + Marfanoid habitus

Quite specific, limited number of alternative diagnoses

→PP4_Supporting

Table 1: Criteria for Marfan Syndrome Diagnosis from Revised Ghent Criteria

In the absence of family history:
(1) Ao (Z ≥2) AND EL=MFS
(2) Ao (Z ≥2) AND FBN1=MFS
(3) Ao (Z ≥2) AND Syst (≥7 pts)=MFS
(4) EL AND FBN1 with known Ao=MFS
EL with or without Syst AND with a FBN1 not known with Ao or no FBN1=ELS
Ao (Z <2) AND Syst (≥5 with at least one skeletal feature) without EL=MASS
MVP AND Ao (Z <2) AND Syst (<5) without EL=MVPS
In the presence of family history:
(5) EL AND FH of MFS (as defined above)=MFS
(6) Syst (≥7 pts) AND FH of MFS (as defined above)=MFS
(7) Ao (Z ≥2 above 20 years old, ≥3 below 20 years) + FH of MFS=MFS

Ao = aortic diameter at the sinuses of valsalva above indicated Z-score or aortic root dissection; EL = ectopia lentis; ELS = ectopia lentis syndrome; FBN1 = fibrillin-1 mutation; FH = family history; MASS = mitral valve prolapse, borderline (Z <2) aortic root dilatation, striae, skeletal findings phenotype; MFS = Marfan syndrome; MVPS = mitral valve prolapse syndrome; Syst = systemic score; Z = Z-score. Source: Loeys et al, 2010.

Revised Ghent criteria for Marfan Syndrome met (no molecular results)

Highly specific, no significant alternative diagnoses

→PP4_Moderate

Internal adaptation

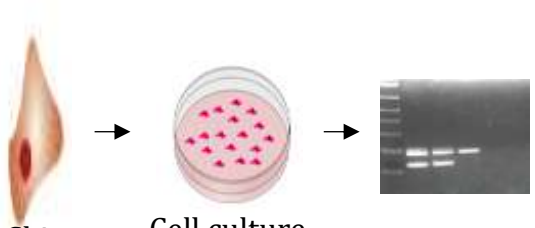
Examples of specific applications of PP4 by the ClinGen WG at https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/

CRITERIA RELATED TO FUNCTIONAL ASSAYS (PS3/BS3 or PVS1_Strength/BP7)

Variants predicted to alter the splicing

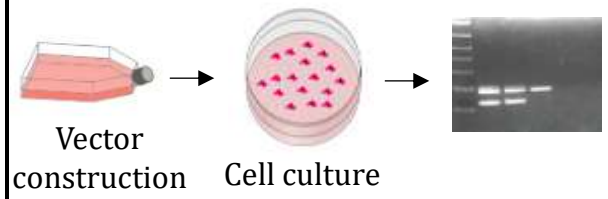
Missense/synonym variants close to the D/A sites
Intronic variants not in the D/A sites
Intronic variants in the D/A sites with a downgraded PVS1 criterion

Tissues expressing the gene available



RT-PCR/WTS analysis

Tissues expressing the gene NOT available
or
Patient dead



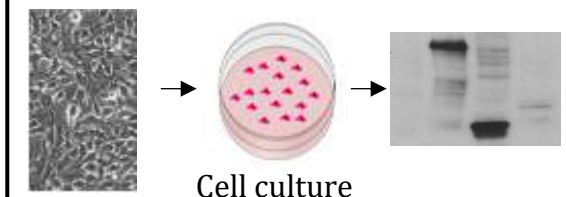
Minigene reporter assay

Structural variants (missense/in-frame indels)

Tissues expressing the gene available

***Ex vivo* cellular studies**

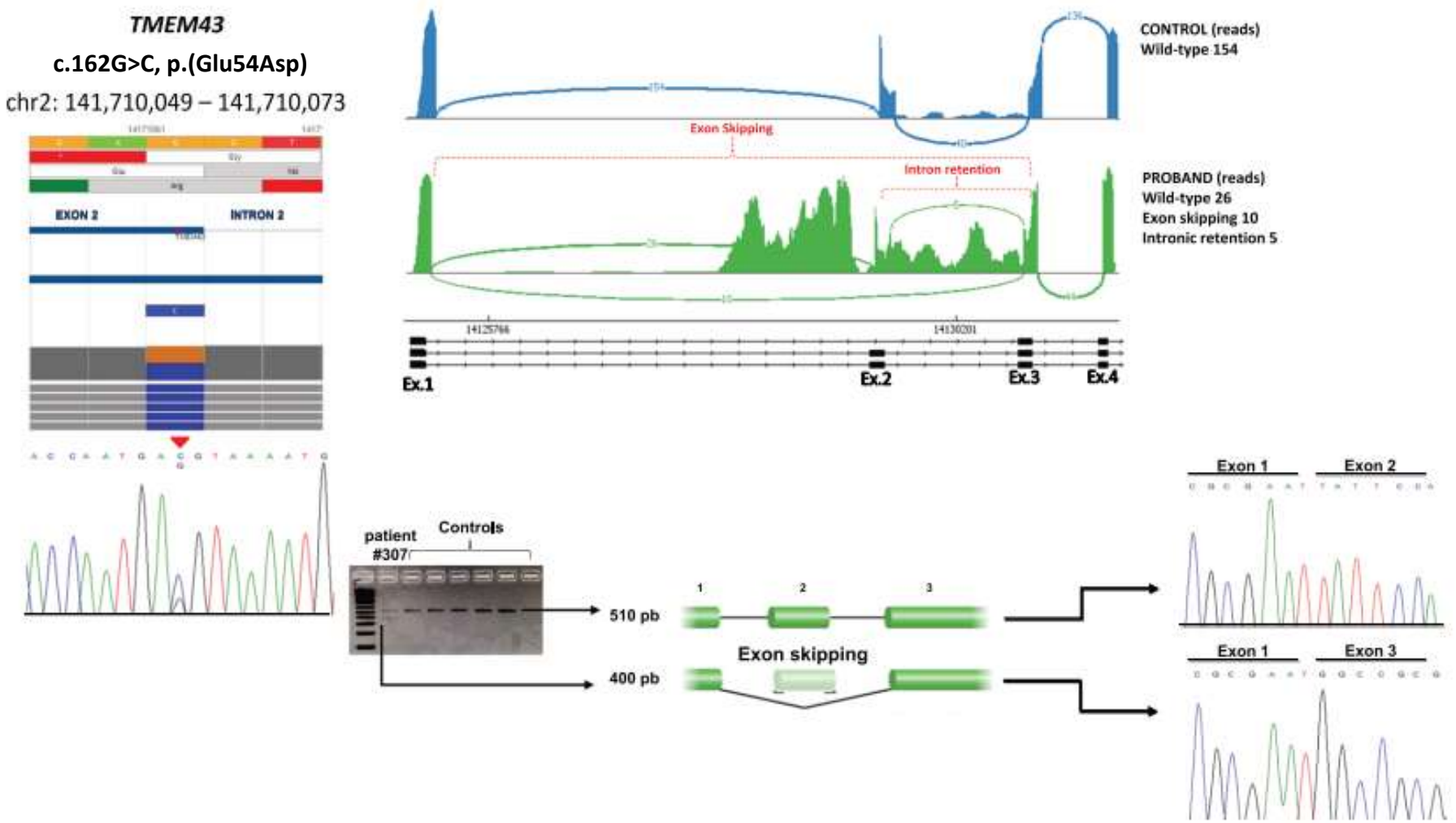
Tissues expressing the gene NOT available
or
Patient dead



***In vitro* cellular studies**

SPLICING VARIANTS: TRASCRIPTOMICS ON PERIPHERAL BLOOD

Case study: a 81-year-old man with arrhythmogenic cardiomyopathy; family history not contributory



Clinical interpretation (VUS):
PM2_Moderate, PP3_Supporting

Clinical interpretation (LP):
PVS1(strength)_Strong, PM2_Moderate

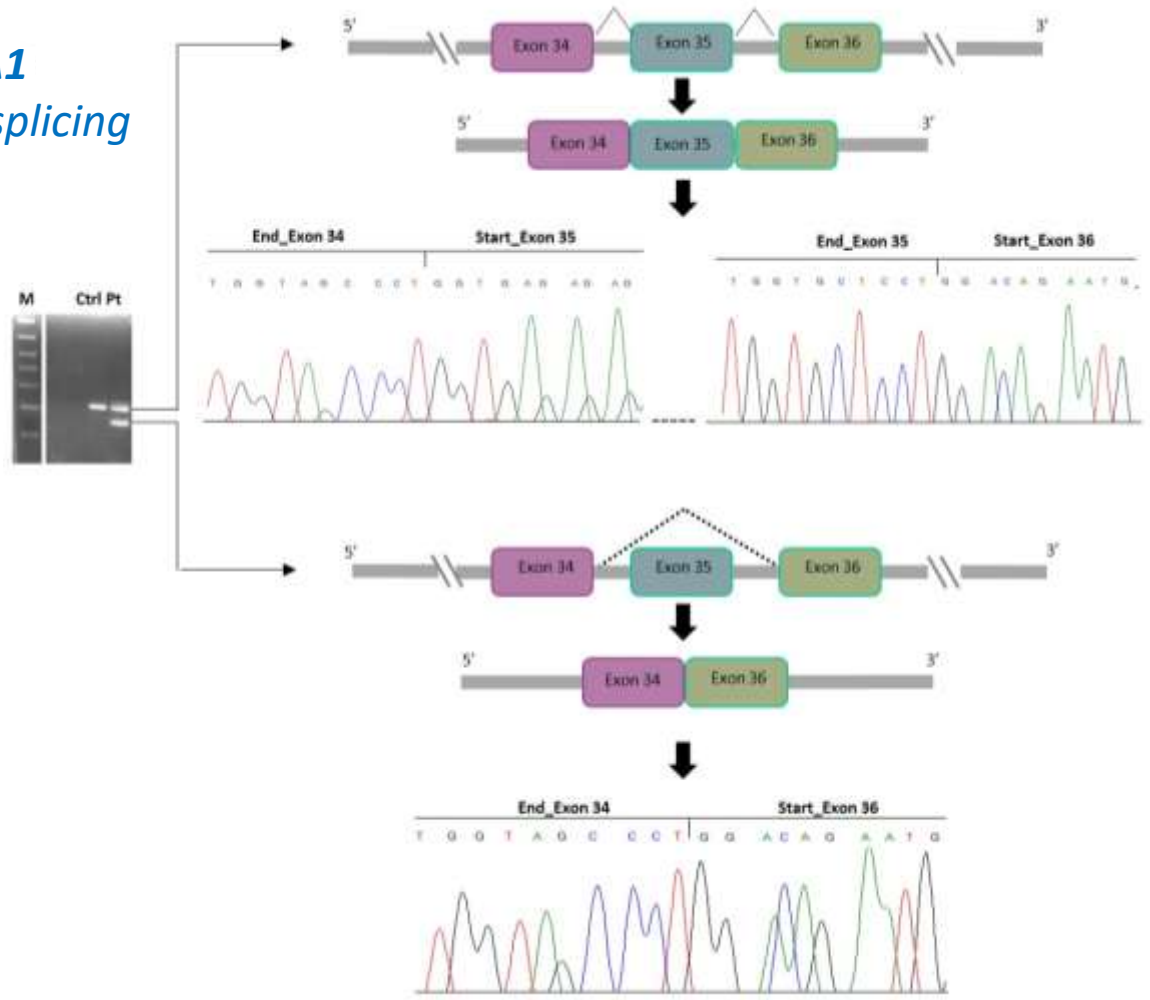
(Castori et al., J Hum Genet 2025)

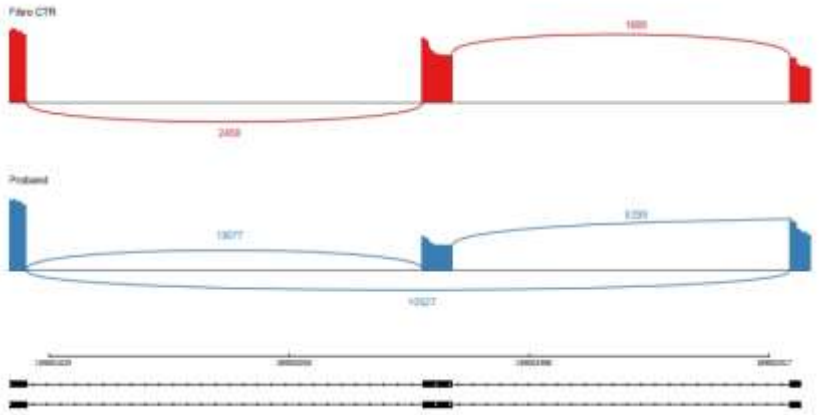
SPLICING VARIANTS: TRASCRIPTOMICS ON FIBROBLASTS

Case study: clinical suspicion of vascular EDS, parent unavailable, a single intronic variant detected at NGS

c.2445+5G>C in *COL3A1*

Predicted altering the splicing





Clinical interpretation (VUS):

PM2_Moderate, PP2_supporting, PP3_Supporting, PP4_Supporting

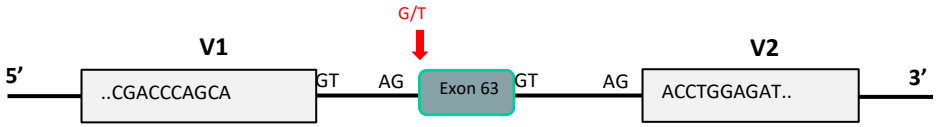
Clinical interpretation (LP):

PM4_Strong, PM2_Moderate, PP2_Supporting, PP4_Supporting

(Leone et al., Hum Genet 2023)

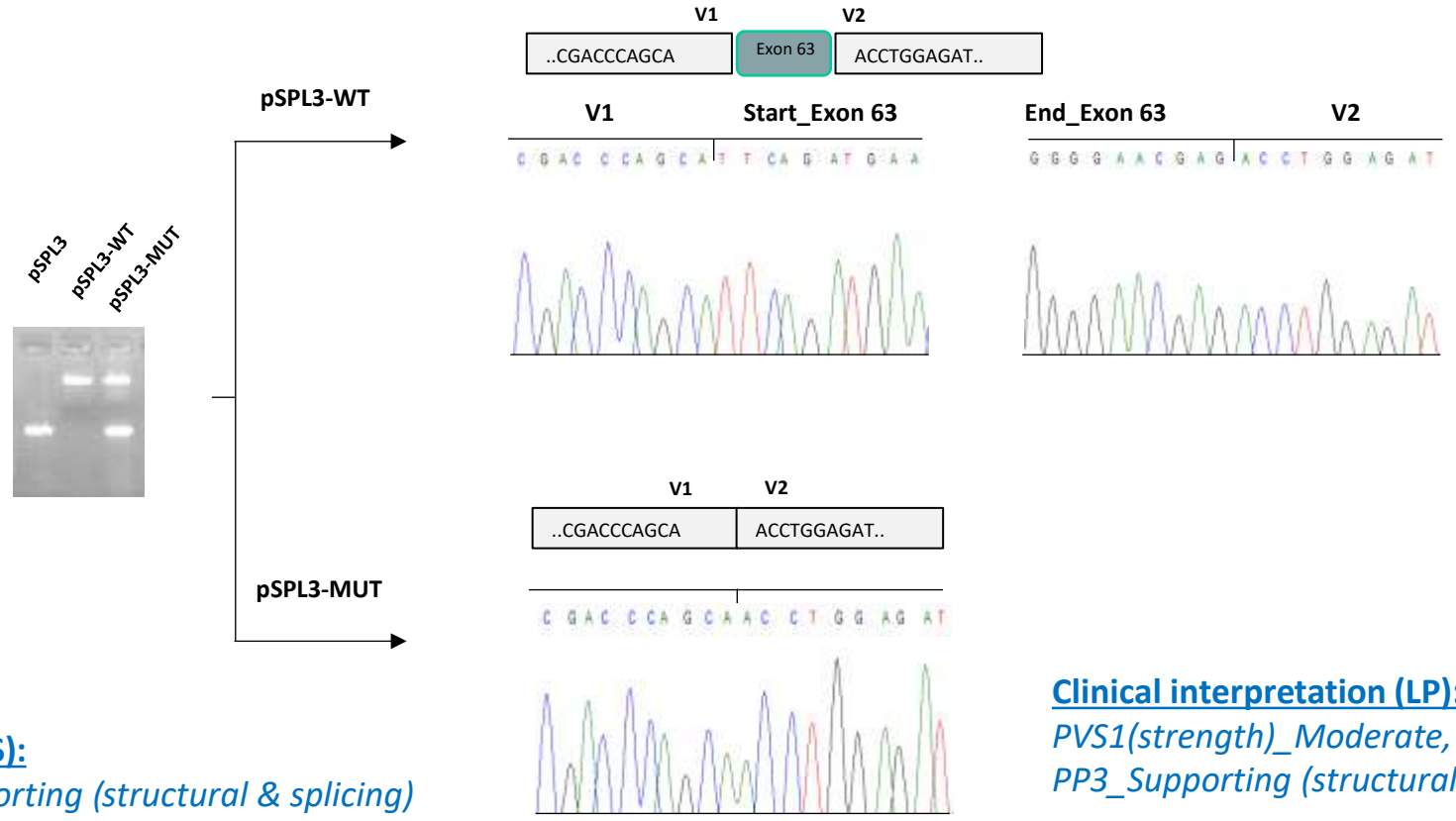
SPLICING VARIANTS: MINIGENE REPORTER ASSAY

Case study: EDS of unknown type referred from an external center, altered EMG and reduced muscle strength by reverse phenotype after molecular testing, unavailability for skin biopsy



c.8753G>T in *COL12A1*

Predicted both p.(Gly2918Val) and splicing



Clinical interpretation (VUS):
PM2_Moderate, PP3_Supporting (structural & splicing)

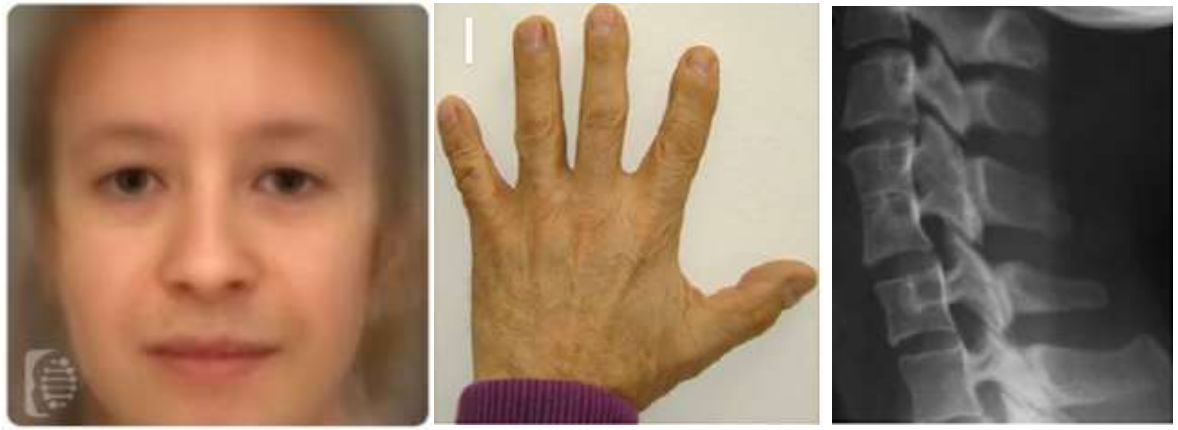
Clinical interpretation (LP):
PVS1(strength)_Moderate, PM2_Moderate, PP3_Supporting (structural), PP4_Supporting

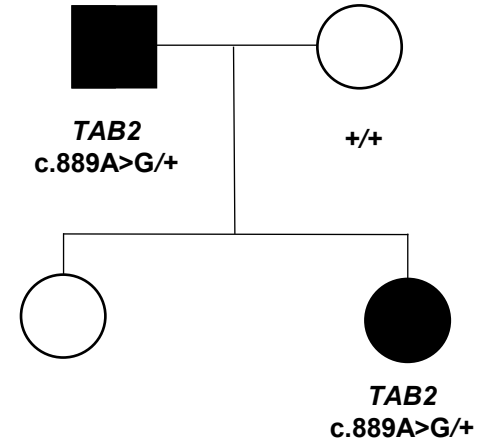
(Leone et al., Hum Genet 2023)

STRUCTURAL VARIANTS (MISSENSE): IN VITRO STUDIES

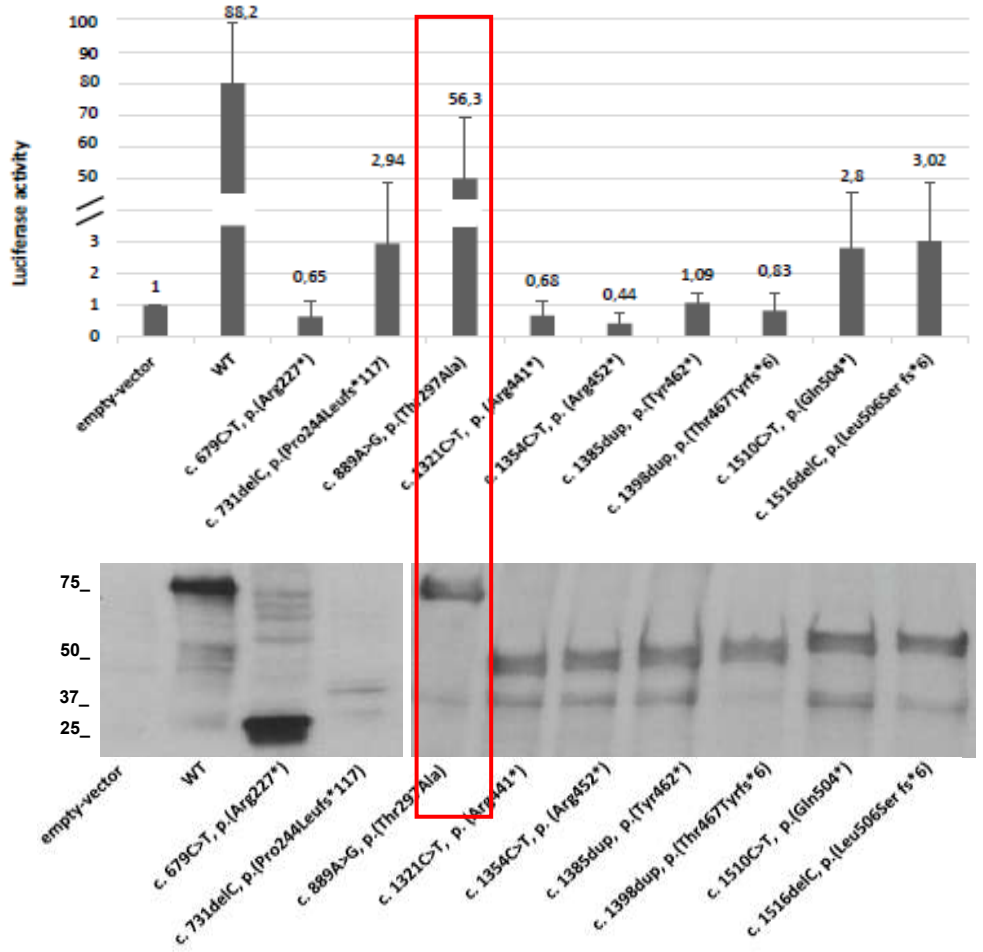
Case study: a family with a *TAB2* private missense variant falling in the mutational hotspot

TAB2-related cardio-facial-cutaneous-articular syndrome





Luciferase assay (plasmids)



Clinical interpretation (VUS):
PM2_Moderate, PP3_Supporting

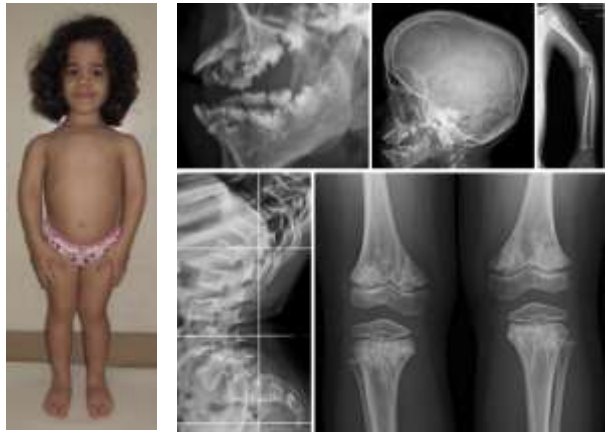
Clinical interpretation (LP):
PS3_Strong, PM2_Moderate

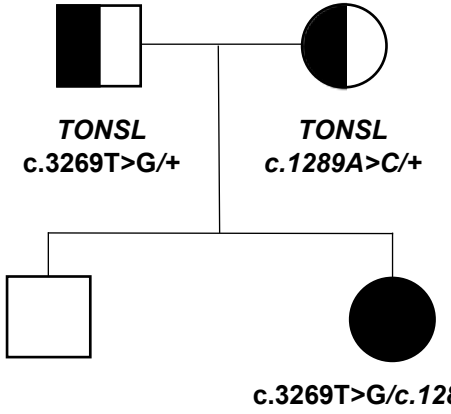
(Micale et al., Genet Med 2022)

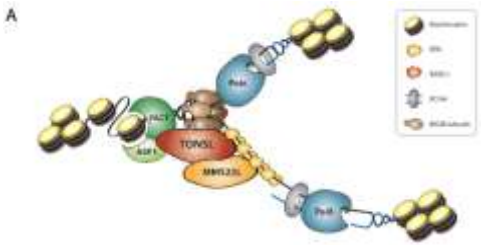
STRUCTURAL VARIANTS (MISSENSE): *EX VIVO* STUDIES

Case study: radiographic diagnosis of SPONASTRIME dysplasia, two missense VUS in *TONSL* at re-analysis of the ES data after the publication of the identification of the causative gene

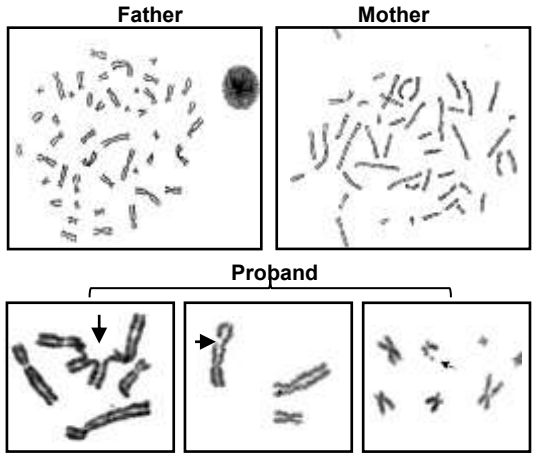
SPONASTRIME dysplasia (*TONSL*)





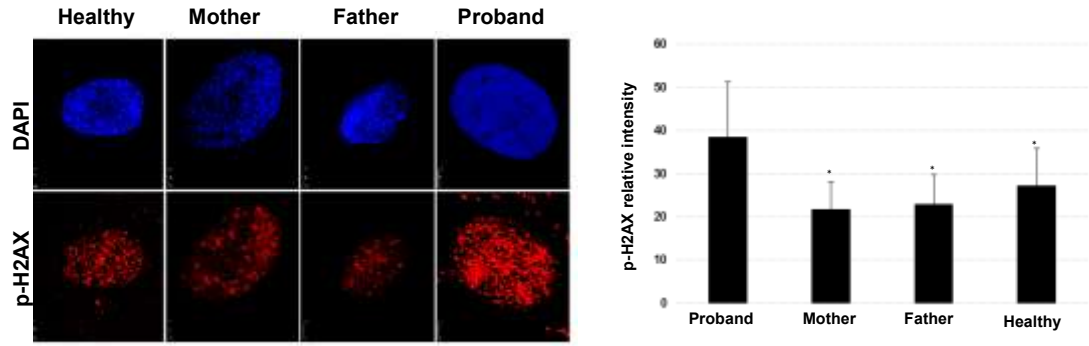


Metaphases from skin fibroblast cellls



Bi-allelic TONSL variants results in genome instability and DNA damage.

Skin fibroblast cells immunocolored with p-H2AX



Clinical interpretation (VUS):
PM2_Moderate, PP3_Supporting, PP4_Supporting

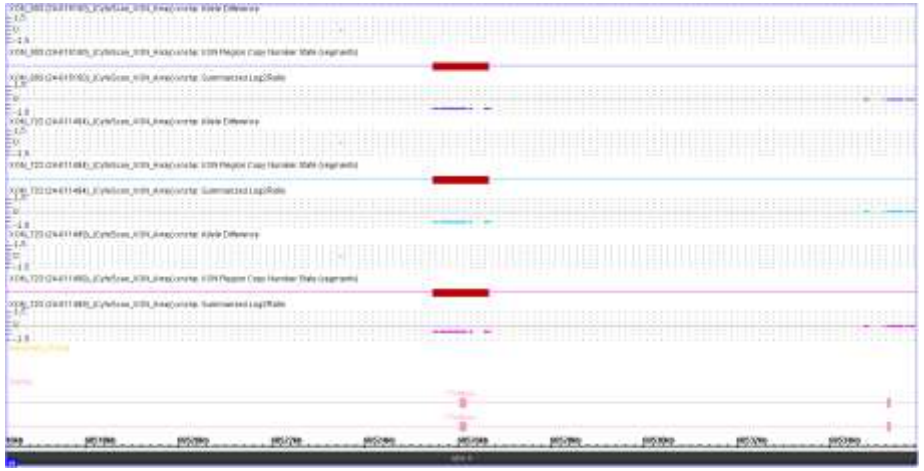
Clinical interpretation (LP):
PS3_Strong, PM2_Moderate, PP4_Supporting

(Micale et al., Hum Mol Genet 2020)

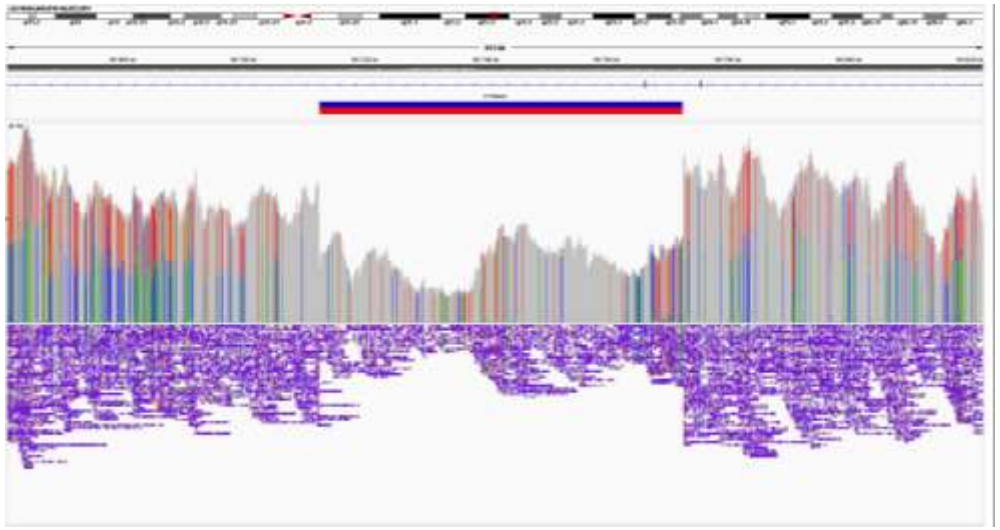
TECHNOLOGICAL INTEGRATION TO SOLVE COMPLEX CASES

Case study: familial recurrence of left ventricular non-dilated cardiomyopathy, negative ‘short reads NGS’

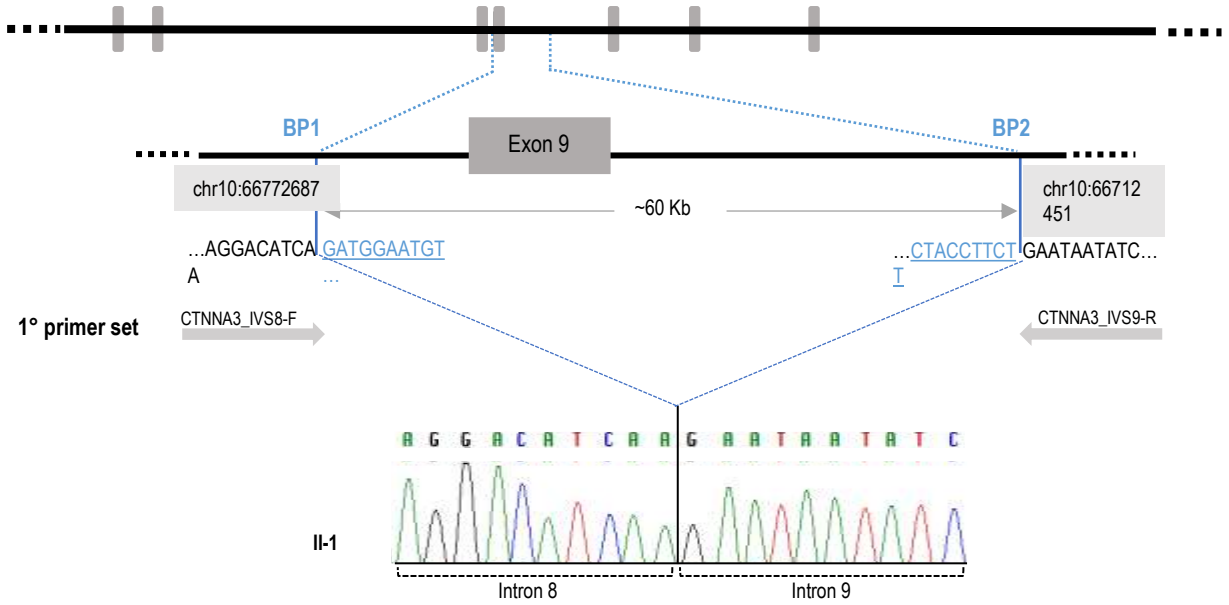
Detection of a single-exon deletion in *CTNNA3* by XONarray



Refinement by Nanopore ‘long reads’ NGS

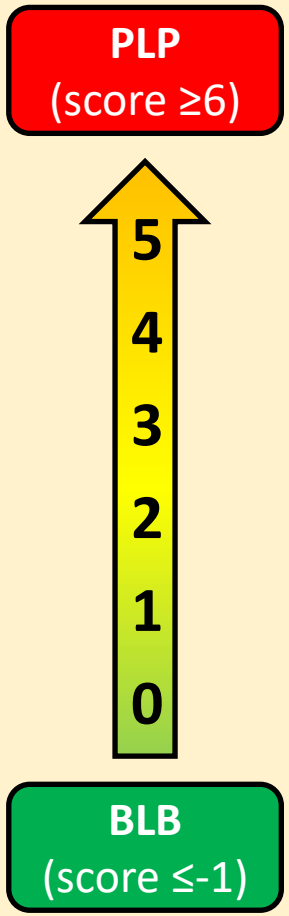


Confirmation for clinical use by Sanger sequencing



PERSPECTIVES FOR SUB-TIERING VARIANTS OF UNCERTAIN SIGNIFICANCE

BAYESIAN SCORE SYSTEM



COMBINATIONS OF CRITERIA

VUS in favour of pathogenicity: rare variants with multiple pathogenicity criteria, but not enough for the PLP status, and none benignity criteria

VUS in favour of benignity: rare variants with benignity criteria, not enough for the BLB status, and not any other pathogenicity criteria

Neutral VUS: rare variants without any other criteria

VUS with conflicting interpretation of data: rare variants with a combination of pathogenicity and benignity criteria

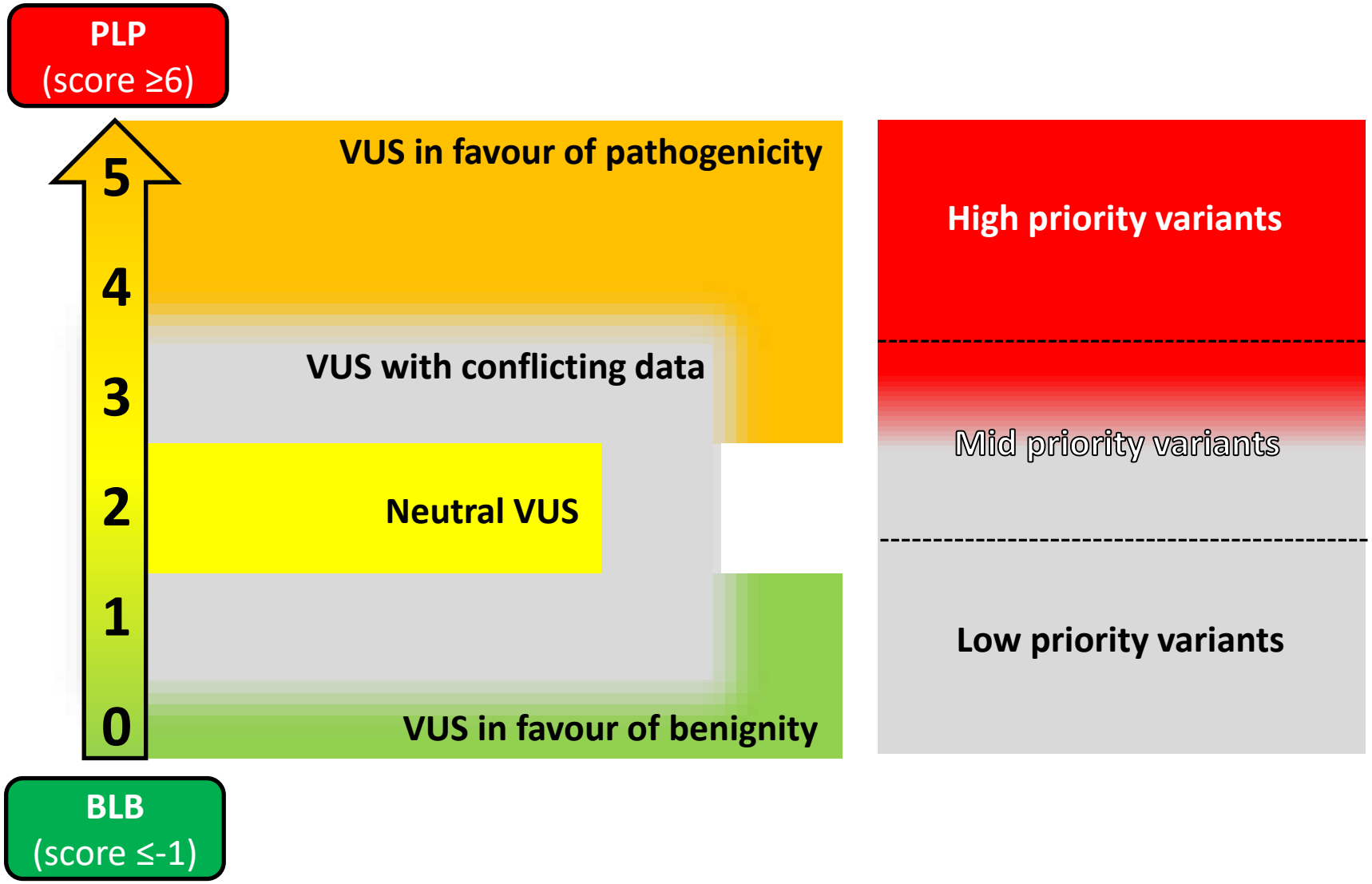
ACTIONABILITY

Is there a reasonable number of actions that, if addressed, might change a VUS into a PLP variant?

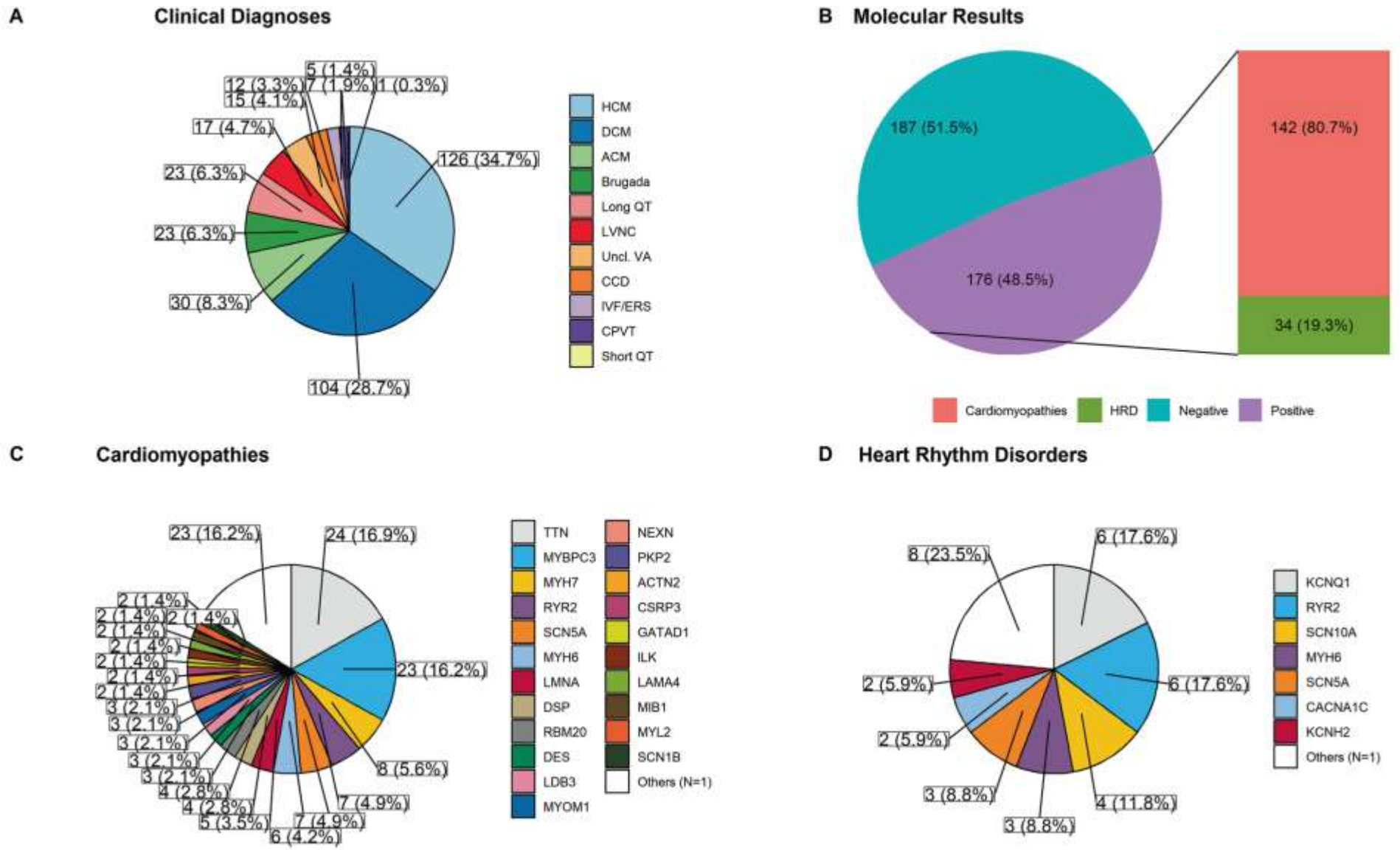
1. *Segregation*
2. Phenotype revision
3. Functional data
4. Phase determination

- **High priority**
- **Low priority**

PERSPECTIVES FOR SUB-TIERING VARIANTS OF UNCERTAIN SIGNIFICANCE

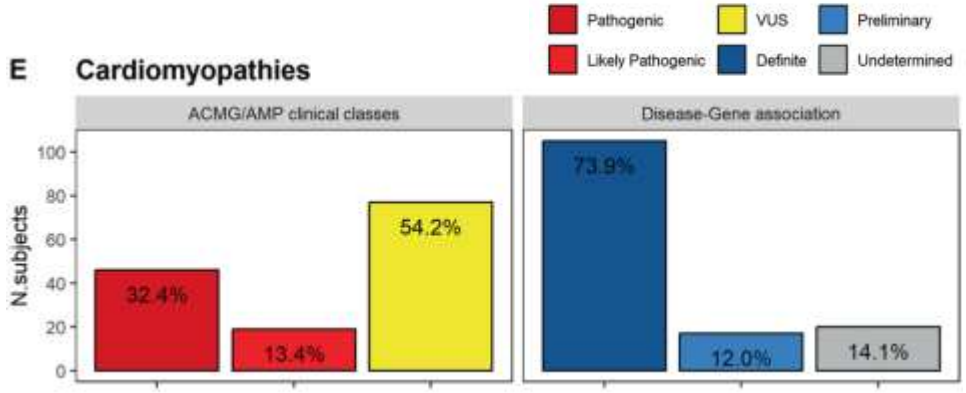


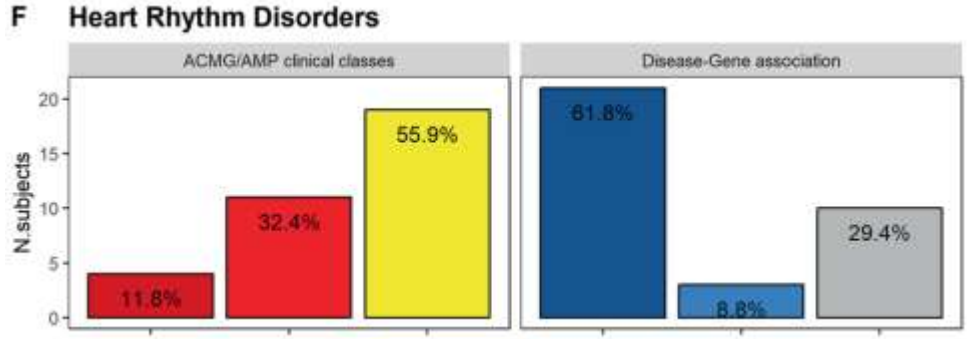
VUS SUB-TIERING IN CARDIOGENETICS: A PILOT STUDY ON 363 PEDIGREES

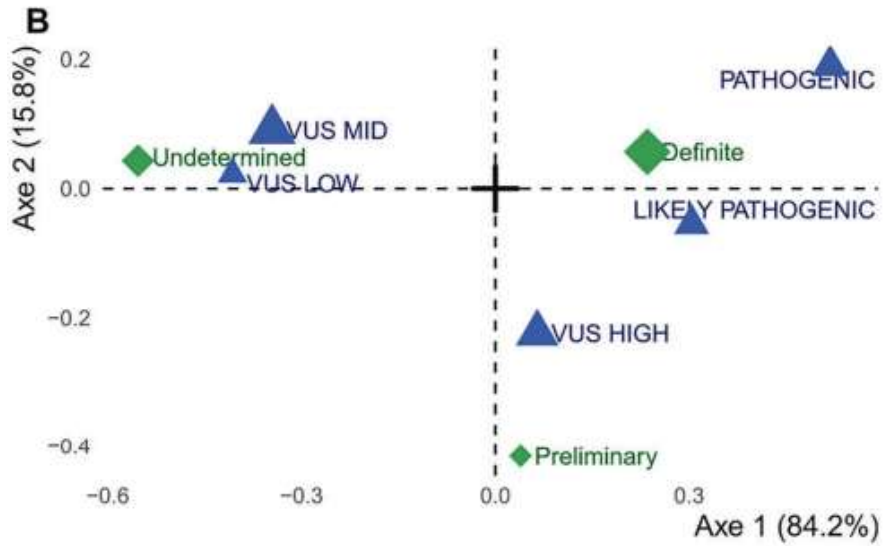


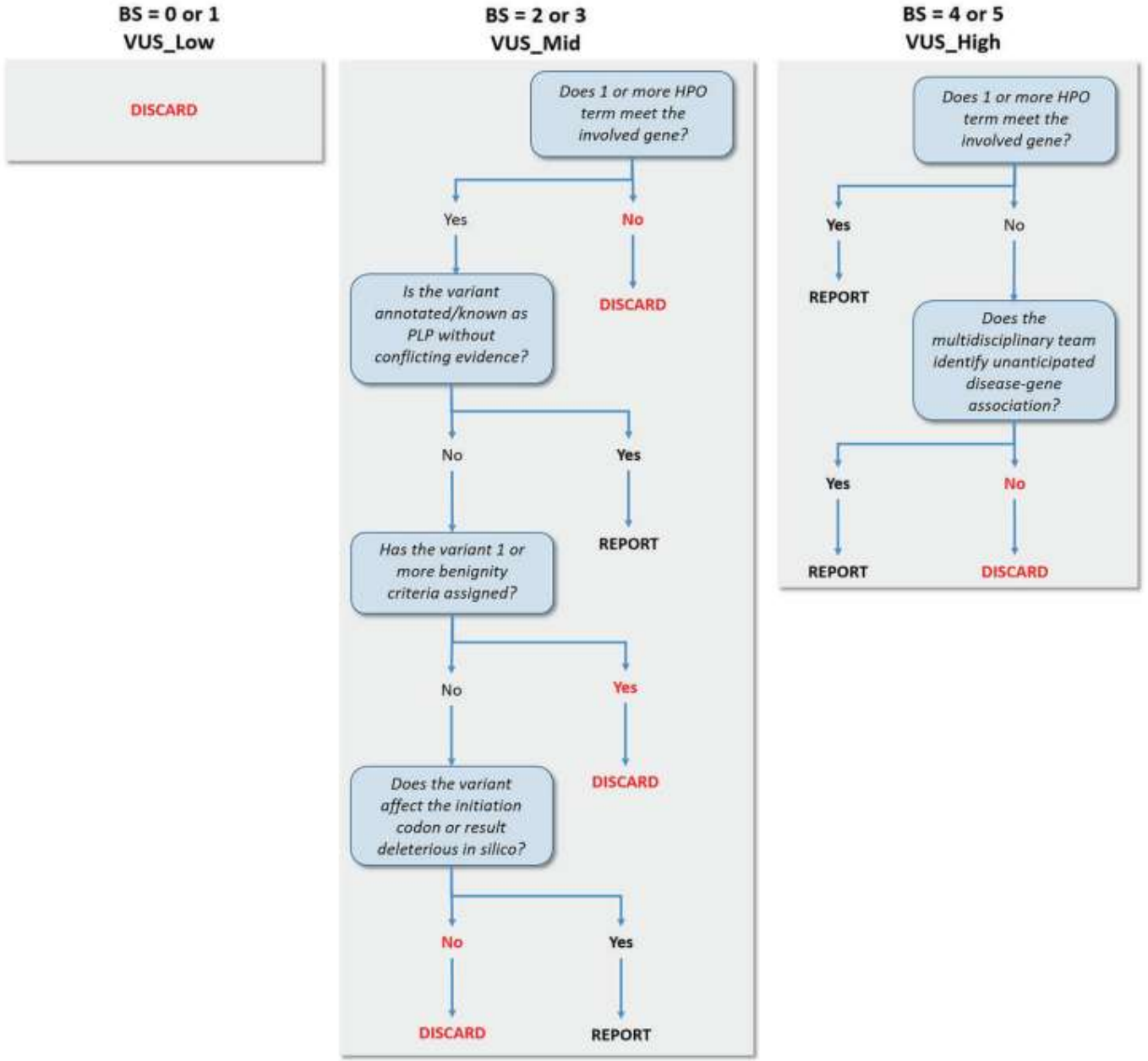
(Castori et al., J Hum Genet 2025)

VUS SUB-TIERING IN CARDIOGENETICS: A PILOT STUDY ON 363 PEDIGREES



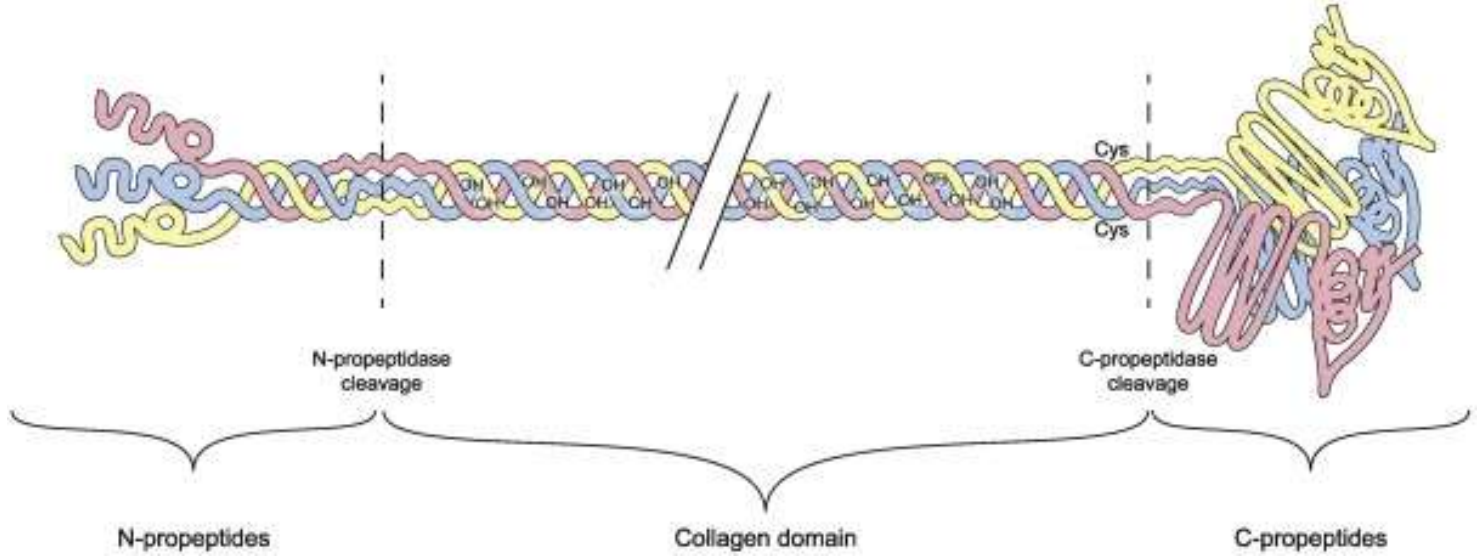






(Castori et al., J Hum Genet 2025)

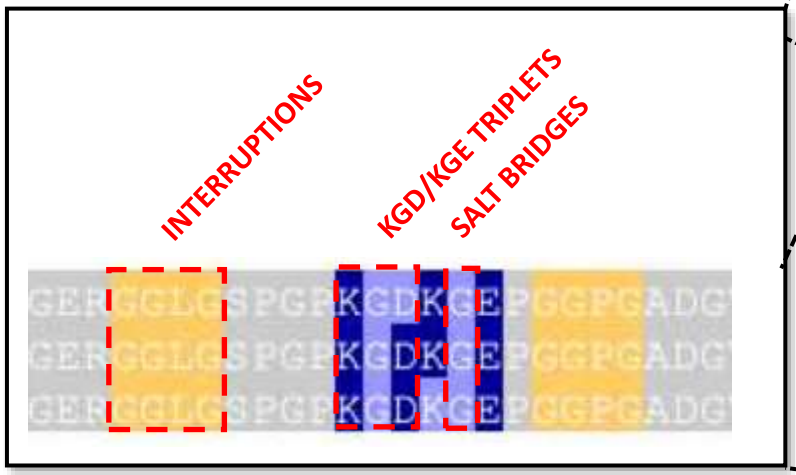
VARIANT INTERPRETATION IN VASCULAR EHLERS-DANLOS SYNDROME



- ✓ **Initiation codon variants** (→ ~ *null alleles*)
- ✓ **Small in-frame indels in the triple helical domain** (→ ~ *splicing variants*)
- ✓ **Stoploss variants** (→ ~ *C-propep. variants*)

?

SPLICING* ALLELES (TRIPLE HELICAL DOM.)
Highest cardiovascular risk
Caveat: verify *(in silico/vitro)* prediction of in-frame del/dup



GLY SUBSTITUTIONS (TRIPLE HELICAL DOM.)
Typical cardiovascular risk
Caveat 1: verify that the substitution does not fall within the triple helix interruptions (*Malcor et al., 2025*)
Caveat 2: Gly→Cys/Ser/Ala might associate with **milder** phenotypes (*Zschocke et al., 2024*)

OTHER SUBSTITUTIONS (TRIPLE HELICAL DOM.)
Variable cardiovascular risk
Glu→Lys likely associated with **typical** cardiovascular risk (*Ghali et al., 2019*)
Caveat for other missense changes:
- ✓ Family segregation
- ✓ Involvement of **KGE/KGD** triplets or salt-bridges (*Malcor et al., 2025*)

C-PROPEPTIDE VARIANTS
Lower cardiovascular risk (*Frank et al., 2015; Stembridge et al., 2025*)
Caveat: the number of published cases is limited
- ✓ Family segregation

***NULL* (POINT) ALLELES**
Lower cardiovascular risk (penetrance ~50%)
Caveat: verify exon skipping with an eventual in-frame del/dup for variants falling within the triple helical domain

WHOLE GENE DELETIONS (2q32 microdeletion)
Lowest cardiovascular risk (*Green et al., 2025*)
Usually found in people with ID/epilepsy
Caveat: the number of published cases is limited

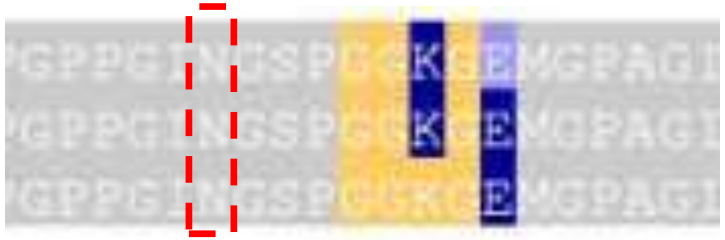
***: variants falling in canonic splice sites (±1,2), intronic and coding (missense and synonym) variants falling in non-canonic splice sites (polypirimidine tract, position +5, etc)**

VARIANT INTERPRETATION IN VASCULAR EHLERS-DANLOS SYNDROME

My name is and I'm a genetic counselor from Medical Center in the . We have a patient who meets hypermobile EDS clinical criteria and genetic testing returned with a variant of uncertain significance in COL3A1 c.1165A>T (p.Asn389Tyr). There has been 2-3 individuals reported with this variant who had

Some hypermobility features and internal carotid artery dissection in their 40-50s. Our patient hasn't had full cardiovascular evaluation yet. I'm just curious if you have seen this variant in your database and patient populations reported the in the paper "Specifications and validation of the ACMG/AMP criteria for clinical interpretation of sequence variants in collagen genes associated with joint hypermobility".

Thank you and I look forward to hearing from you!



ClinVar
Clinically relevant variation

- ✓ 7 Submissions VUS ★☆☆☆
- ✓ 1 Submission Benign ★☆☆☆
- ✓ 1 Submission Likely Benign ★☆☆☆

SNV: 2-188994053-A-T(GRCh38) Copy variant ID

	Exomes	Genomes	Total
Filters	Pass	Pass	
Allele Count	357	9	366
Allele Number	1461808	152290	1614098
Allele Frequency	0.0002442	0.00005910	0.0002268
Grpmax Filtering AF (95% confidence)	0.0002889	0.00005842	0.0002790
Number of homozygotes	0	0	0

Estimated disease frequency = 1/20,000 (0.00005) to 1/50,000 (0.00002)

VARIANT INTERPRETATION IN VASCULAR EHLERS-DANLOS SYNDROME

mi chiamo e sono un medico specialista in Genetica Medica, attualmente in Servizio presso la Genetica Medica di .
La disturbo per chiederle un parere circa la variante c.1996G>A p.(Gly666Ser) del gene *COL3A1* (NM_000090.3).
Tale variante è stata identificata presso altro Centro in una paziente con situs inversus ed è stata ereditata dal padre che, come la paziente ed il resto della famiglia, non mostra segni di vEDS. Le caratteristiche molecolari la fanno classificare come C4 ma in effetti la storia familiare è completamente muta (la paziente ha anche avuto una gravidanza a termine senza complicanze).
Non trovo in letteratura dati di altri pazienti descritti con tale variante e sono quindi a chiederle se l'avete mai identificata e se concorda con tale classificazione poichè, in tal caso, la cercherei anche negli altri familiari, compresi i minori.
La ringrazio anticipatamente per il suo tempo e porgo cordiali saluti,

ClinVar
Clinically relevant variation

- ✓ 1 Pathogenic ★☆☆☆
- ✓ 8 Likely Pathogenic ★☆☆☆

Classification	Pathogenic/Likely pathogenic
★★☆☆	9 out of 9 submissions contributed t

SNV: 2-188998692-G-A(GRCh38) Copy variant ID

	Exomes	Genomes	Total
Filters	Pass	No variant	
Allele Count	12	0	12
Allele Number	1461616	152226	1613842
Allele Frequency	0.000008210		0.000007436
Grpmax Filtering AF (95% confidence)	0.000005310		0.000005000
Number of homozygotes	0		0

Estimated disease frequency = 1/20,000 (0.00005) to 1/50,000 (0.00002)

THANKS FOR YOUR ATTENTION

GRAZIE PER L'ATTENZIONE