ClinGen Cardiomyopathy Variant Curation Expert Panel (CMP-VCEP)

Updated ACMG/AMP Classification Rules for Cardiomyopathy

PS4 Example Scenarios:

General Example - Variant A			
Comparison	**Calculation (# per Genotype)**	**OR [95% CI]**	**Rule Strength**
Clinical lab: 22 in 7,437 gnomAD (Total): 1 in 120,591	**p/q** **p/p** *Cases (Clinical lab)* 22 7,415 *gnomAD (Total)* 1 120,590	358 [**48**-2,655]	**STRONG**
Literature: 13 in 9,162 gnomAD (Total): 1 in 120,591	**p/q** **p/p** *Cases (Literature)* 13 9,149 *gnomAD (Total)* 1 120,590	171 [**22**-1,310]	**STRONG**

Case data (comparisons) are presented as number of individuals in whom a heterozygous variant (genotype p/q) was detected and the total number of individuals in the cohort. For controls, gnomAD (Genome Aggregation Database) is used. The gnomAD Allele count (AC) is considered to represent the number of individuals in whom the variant was detected (assuming there are no homozygous occurrences of the variant); the gnomAD allele number (AN) is divided by two to represent the total number of individuals for whom data is available at the site of the variant. gnomAD (Total) combines data from all populations represented in gnomAD. OR=Odds ratio. 95% CI=95% confidence intervals. The lower 95% CI of the OR (the value considered when selecting PS4 criteria strength) is highlighted in bold. Rule strengths: Supporting (lower 95% CI of the OR ≥5); Moderate (lower 95% CI of the OR ≥10); Strong (lower 95% CI of the OR ≥20).

Variant A has been detected in a clinical laboratory in 22 out of 7,437 cases referred for HCM testing. It has been described in the literature in 13 out of 9,162 HCM cases. It is listed in gnomAD at a total frequency of 1/241,182 alleles. The population diversity represented in gnomAD is considered to (broadly) reflect the diversity of the referral population of the case cohorts. Since the lower bound of the 95% confidence interval around the odds ratio (OR) estimates in both case vs gnomAD analyses are greater than the STRONG threshold of ≥20, PS4 should be applied at STRONG.

Selecting a Control Cohort - Variant B			
Comparison	**Calculation (# per Genotype)**	**OR [95% CI]**	**Rule Strength**
Clinical lab: 12 in 5,792 gnomAD (Total): 1 in 119,295	**p/q** **p/p** *Cases (Clinical lab)* 12 5,780 *gnomAD (Total)* 1 119,294	248 [**32**-1,905]	**STRONG**
Literature: 15 in 7,873 gnomAD (Total): 1 in 119,295	**p/q** **p/p** *Cases (Literature)* 15 7,858 *gnomAD (Total)* 1 119,294	227 [**30**-1,724]	**STRONG**
Clinical lab: 12 in 5,792 gnomAD (European): 1 in 61,865	**p/q** **p/p** *Cases (Clinical lab)* 12 5,780 *gnomAD (European)* 1 61,864	128 [**17**-988]	**MODERATE**
Literature: 15 in 7,873 gnomAD (European): 1 in 61,865	**p/q** **p/p** *Cases (Literature)* 15 7,858 *gnomAD (European)* 1 61,864	118 [**16**-894]	**MODERATE**

Case data (comparisons) are presented as number of individuals in whom a heterozygous variant (genotype p/q) was detected and the total number of individuals in the cohort. For controls, gnomAD (Genome Aggregation Database) is used. The gnomAD Allele count (AC) is considered to represent the number of individuals in whom the variant was detected (assuming there are no homozygous occurrences of the variant); the gnomAD allele number (AN) is divided by two to represent the total number of individuals for whom data is available at the site of the variant. gnomAD (Total) combines data from all populations represented in gnomAD. gnomAD (European) data is from the non-Finnish European subpopulation. OR=Odds ratio. 95% CI=95% confidence intervals. The lower 95% CI of the OR (the value considered when selecting PS4 criteria strength) is highlighted in bold. Rule strengths: Supporting (lower 95% CI of the OR ≥5); Moderate (lower 95% CI of the OR ≥10); Strong (lower 95% CI of the OR ≥20).

Variant B has been detected in a clinical laboratory in 12 out of 5,792 cases referred for HCM testing. It has been described in the literature in 15 out of 7,873 HCM cases from predominantly European only cohorts. It is listed in gnomAD at a total frequency of 1 in 238,590 alleles and at a European (non-Finnish) frequency of 1 in 123,730 alleles. It is appropriate to consider both gnomAD Total and European data for these case-control analyses. For both case vs gnomAD Total analyses, the lower bound of the 95% CI around the OR estimates are above the STRONG threshold of ≥20. For both case vs gnomAD (European) analyses, the lower bound of the 95% CI around the OR estimates are in the MODERATE range (≥10 and <20). Since this appears to be a predominantly European variant, case-control analyses using the gnomAD European data may be considered the more appropriate and conservative approach. Therefore, PS4 should be applied at MODERATE.

Comparing Cohort and Available Data - Variant C				
Comparison	**Calculation (# per Genotype)**	**p/q**	**p/p**	**OR [95% CI]**	**Rule Strength**
Clinical lab A: 4 in 2,481 gnomAD (Total): 0 in 116,190	*Cases (Clinical lab A)*	4	2,477	422 [**23**-7,841]	**STRONG**
	gnomAD (Total)	0	116,190		
Clinical lab B: 8 in 5,953 gnomAD (Total): 0 in 116,190	*Cases (Clinical lab B)*	8	5,945	332 [**19**-5,757]	**MODERATE**
	gnomAD (Total)	0	116,190		
Literature: 6 in 5,154 gnomAD (Total): 0 in 116,190	*Cases (Literature)*	6	5,148	293 [**16**-5,208]	**MODERATE**
	gnomAD (Total)	0	116,190		

Case data (comparisons) are presented as number of individuals in whom a heterozygous variant (genotype p/q) was detected and the total number of individuals in the cohort. For controls, gnomAD (Genome Aggregation Database) is used. The gnomAD Allele count (AC) is considered to represent the number of individuals in whom the variant was detected (assuming there are no homozygous occurrences of the variant); the gnomAD allele number (AN) is divided by two to represent the total number of individuals for whom data is available at the site of the variant. gnomAD (Total) combines data from all populations represented in gnomAD. OR=Odds ratio. 95% CI=95% confidence intervals. The lower 95% CI of the OR (the value considered when selecting PS4 criteria strength) is highlighted in bold. Rule strengths: Supporting (lower 95% CI of the OR ≥5); Moderate (lower 95% CI of the OR ≥10); Strong (lower 95% CI of the OR ≥20).

Variant C has been detected in two clinical laboratories in the US. Lab A in 4 out of 2,481 cases referred for DCM testing. Lab B in 8 out of 5,953 cases referred for DCM testing. This variant has been described in four different DCM cohorts in the literature from European, Asian and US populations. These comprise cases referred for DCM genetic testing and clinical cohorts of selected DCM cases. These cohorts are presumed to be non-overlapping; the combined total of occurrences is 6 out of 5,154 DCM cases. Overlap between the clinical laboratories and the cases in the literature cannot be excluded, therefore it was not considered appropriate to combine datasets for analysis.

This variant has not been detected in gnomAD v2.1.1; based on data from a nearby variant it is estimated that the allele number is ~232,380 alleles. The population diversity represented in gnomAD (Total) is considered to (broadly) reflect the diversity of the populations referred for genetic testing used as case cohorts.

The lower bound of the 95% CI around the OR estimates in the Lab A vs gnomAD (Total) analysis is above the STRONG threshold, while the Lab B analysis is just under it as MODERATE. The literature cases vs gnomAD total are also in the MODERATE range.

The assessment also identified some individual DCM case reports with this variant in the literature which do not appear to overlap with the data used in case-control analyses. The variant is also listed by several laboratories in ClinVar as seen in cases, but without clinical data.

There are several key points to consider for assignment of PS4:

1. This variant has not been detected in gnomAF (v2.1.1 or v3.1.2)
2. Phenotype is consistent across multiple cohorts (i.e., all appear to be DCM cases rather than HCM)
3. The variant is enriched in three apparently non-overlapping cohorts (higher moderate to strong range)
4. The criteria for PS4 rule application are intentionally conservative.

Therefore, given the above evidence it would be acceptable to apply PS4 at STRONG. However, it would also be appropriate to apply PS4 at MODERATE if a more conservative approach is preferred given the specific evidence presented and clinical judgement.