SVI Recommendation for in *trans* Criterion (PM3) - Version 1.0

The Sequence Variant Interpretation (SVI) Working Group proposes a point-based system to determine the strength of in *trans* observations (ACMG/AMP criterion PM3) based upon variant phasing and classification of the variant occurring on the other allele. Additionally, SVI recommends a revision to the criterion definition to indicate this evidence should only be applied if the individual is affected:

SVI revision to PM3: For recessive disorders, detected in *trans* with a pathogenic ***or likely pathogenic*** variant ***in an affected patient***

To determine the appropriate strength level to apply for in *trans* occurrence(s), each proband is awarded a point value based upon phasing of the two variants in question (confirmed in *trans* versus unknown) and classification of the variant on the other allele (Table 1). The combined point value of all proband occurrences is then summed and compared to Table 2 to determine the applicable evidence strength level. For example, if assessing *PAH* variant NM_000277.3:c.1208C>T (p.Ala403Val) and the variant was confirmed in *trans* with Likely pathogenic variant c.1301C>A (p.Ala434Asp) in one proband (1.0 points; Table 1) and confirmed in *trans* with Pathogenic variant c.331C>T (p.Arg111Ter) in another proband (1.0 points, Table 1), then PM3 at the Strong strength level (PM3_Strong) is applicable (2.0 points total; Table 2).

Table 1. Points awarded per in *trans* proband

Classification/Zygosity of other variant [1]	Points per Proband	
	Confirmed in *trans*	Phase unknown
Pathogenic or Likely pathogenic variant	1.0	0.5 (P) 0.25 (LP)
Homozygous occurrence *(max point 1.0)*	0.5	N/A
Uncertain significance variant *(max point 0.5)*	0.25	0.0

[1]All variants should be sufficiently rare (meet PM2 specification); P - Pathogenic; LP - Likely pathogenic

Table 2. Recommendation for determining the appropriate evidence strength level for PM3

PM3_Supporting	PM3	PM3_Strong	PM3_VeryStrong
0.5	1.0	2.0	4.0

Considerations:

- **Allele Frequency** - Application of PM3 is contingent on the allele frequency of the variant being assessed and the variant presumably on the other allele both being sufficiently rare (meets PM2 threshold). This contingency is to avoid incorrect application of PM3 to high frequency variants that are likely to occur in *trans* with P/LP variants based on frequency.

- **Phasing** - If the phase cannot be determined, it is recommended that at least two different LP/P variants (depending on classifications) are needed to equal the weight of one LP/P co-occurrence confirmed in *trans*.
    - In confirmation of phasing, if only one parent is tested and found to carry one allele, variants can be counted as in *trans*. For example, assessing PAH variant c.601C>T (p.His201Tyr) and variant was identified in PKU proband who also carries known pathogenic variant c.734T>C (p.Val245Ala). Only the mother is available for testing and the mother only carries c.734T>C (p.Val245Ala) variant, then variants can be considered in *trans*.

- **Classification** - Probands should be weighted less when the variant on the other allele is of uncertain significance and rare (meets PM2); however, weight may vary by gene size as larger genes are more likely to have a second variant by chance (default 0.25 points). If the variant on the other allele is classified as P or LP, weighting depends on phasing (see *Phasing* above), with P/LP being weighted equally if confirmed in *trans* and different point values per proband if phasing is unknown (0.5 points and 0.25 points, respectively). To avoid circularity, in all instances (phasing confirmed or unknown), the classification of the variant on the other allele should not use evidence from the variant being interrogated.

- **Homozygous occurrences** - For homozygous occurrences, the default weight is dropped to 0.5 points, as a rare homozygous occurrence may be due to consanguinity. A recommended max of 1.0 points of all homozygous cases is suggested to prevent overclassification of homozygous occurrences in the absence of additional data.