Evaluating the evidence - Functional data

Evidence	1. Functional studies	2. Functional domains or mutational hotspots	3. Constraint score
ACMG code	**PS3** **BS3**	**PM1**	**PP2**
Details	**PS3:** if well-established functional studies support a deleterious effect of a variant on the gene or gene product. May be downgraded to moderate or supporting, depending on the strength of the evidence. **BS3:** if well-established functional studies show no deleterious effect.	**PM1:** only for missense variants that are located either in a critical functional domain without benign missense variation or in a mutational hotspot (i.e. a location where pathogenic variants have been observed at a greater frequency than expected). May be upgraded to Strong for variants affecting certain very specific residues that are known to be critical to protein structure or function e.g. variants in the triple helical domain of *COL1A1* where glycine substitutions are known to affect the protein structure.[3] May be downgraded to Supporting for variants in the functional domain of a gene with *some* (a very low level) of benign missense variation (or kept at moderate if the gene is recessive).	**PP2**: only for missense variants in significantly missense-constrained genes (defined in gnomAD as a Z score >3.09) in which missense variants are a common mechanism of disease.
Comments	For detailed guidance on how to apply the **PS3** evidence criterion, see ACGS update.[1] Functional studies should be assessed according to Clin Gen SVI guidance on the application of **PS3/BS3**.[2]	For further examples of specific residues in genes for which **PM1** can be upgraded to Strong, see ACGS update.[1]	For more about constraint scores and how to use them, see Step 2.10.

References

1. Ellard S, Bable E, Callways A, et al. ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020. *AGCS*. 2020

2. Brnich, S.E., Abou Tayoun, A.N., Couch, F.J. *et al.* Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome Med* **12,** 3 (2020). https://doi.org/10.1186/s13073-019-0690-2.

3. Marini JC, Forlino A, Cabral WA, et al. Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans. Hum Mutat. 2007;28(3):209-221. doi:10.1002/humu.20429