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CORRESPONDENCE

Correspondence on "Computational prediction of protein subdomain stability in *MYBPC3* enables clinical risk stratification in hypertrophic cardiomyopathy and enhances variant interpretation" by Thompson et al.

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Hypertrophic cardiomyopathy (HCM), the most common genetic disease of the myocardium, is a significant cause of adverse events ranging from thromboembolisms to arrhythmias, heart failure, and sudden cardiac death.[1,2] The discovery that variants in genes coding for sarcomeric proteins cause HCM has been instrumental for the clinical management of affected families.[2] For instance, the identification of a pathogenic gene variant in an HCM patient triggers cascade family genetic screening that can detect individuals at risk of developing the disease. However, the classification of variants according to their pathogenicity remains the main challenge of cardiovascular genetics in HCM. Indeed, the field is eagerly pursuing methods that can support variant interpretation in the absence of conclusive enrichment and cosegregation data.[2] Missense variants in *MYBPC3*, the most frequently mutated gene in HCM, are a prime target of such new developments.

In this context, we have read with great interest the report by Thompson et al.[3] The authors have applied STRUM, a bioinformatics tool that predicts protein destabilization, to missense variants belonging to cMyBP-C, the protein coded by *MYBPC3*. Their work is based on the assumption that protein destabilization can lead to cMyBP-C loss of function, which is a well-known trigger of HCM. Indeed, cMyBP-C domain destabilization has been observed experimentally in HCM-linked missense variants of cMyBP-C in the past.[4–7] Cleverly, the authors used a set of known pathogenic and benign variants to estimate STRUM sensitivity (32%) and specificity (93%) for the identification of pathogenic missense *MYBPC3* variants. In principle, a low sensitivity value is not surprising since domain destabilization is not the only HCM pathomechanism that can be induced by pathogenic *MYBPC3* missense variants. However, false negatives, i.e., destabilizing variants not captured by STRUM, can also contribute to the observed low sensitivity (see below). On the other hand, STRUM's high specificity supports application to pathogenicity assessment of *MYBPC3* variants. Indeed, the authors propose that a STRUM+ result can provide supporting evidence of pathogenicity (American College of Medical Genetics and Genomics/Association for Molecular Pathology [ACMG/AMP] PP3 criterion). Enticingly, the report also includes results from in silico saturation mutagenesis experiments, which have resulted in a database of all possible STRUM+ variants that geneticists can readily look up to evaluate novel variants as they are identified. However, although the in silico results in combination with clinical risk data from the SHaRe registry are very compelling, the authors sought experimental validation of just a few STRUM predictions. Specifically, they discuss literature data on the expression in primary cardiomyocytes of seven cMyBP-C mutants. Surprisingly, cMyBP-C Trp792Arg and Arg810His, which are STRUM+, incorporate normally in the sarcomere and have normal half lives.[7] These results may indicate that STRUM specificity to detect protein destabilization is not as high as it would be desired. Given the actionable nature of *MYBPC3* variants classified as pathogenic, which can influence clinical management of HCM families, we feel that further experimental assessment of STRUM predictions is needed to define its use in variant interpretation.

Our group has recently determined the stability of 33 mutant cMyBP-C domains recombinantly expressed in bacteria (refs. [6,8,9] and unpublished data). We have used these data to assess STRUM predictions (data available upon request). In our experiments, we have found that extensive destabilization can result in the impossibility to produce recombinant mutant domains. In those cases in which mutant domains can be expressed, we have found that destabilizing variants can perturb domain structure, as assessed by altered far-UV circular dichroism spectra, or induce large drops in melting temperature (>10 °C). Our validation analysis shows that STRUM sensitivity to capture domain destabilization is 60% (9/15 variants), while specificity is 89% (16/18 variants). Hence, we find that STRUM specificity is remarkably high, although the algorithm currently misses 40% of variants causing protein destabilization, potentially contributing to suboptimal sensitivity to capture pathogenic variants as discussed above.

STRUM's ability to predict protein destabilization in domains with no high-resolution structural information (~50% of cMyBP-C domains) can be of great value. When we assess STRUM predictions for domains with known structures, we find that sensitivity increases to 71% (5/7 variants) while specificity remains high (85%, 11/13 variants). The sensitivity of STRUM in the absence of high-resolution structural information drops to 50% (4/8 variants). Specificity in this case is 100%, although this value has to be taken with a grain of salt since only five variants fall in this category. Hence, our data suggest that STRUM sensitivity is higher when applied to domains for which high-resolution structures are available, although predictions appear to be still useful when STRUM is fed with variants targeting domains with unknown structure. All in all, these results stress the need to obtain high-resolution structures of all cMyBP-C domains.

In summary, the strategy proposed by Thompson et al. is a significant step forward in the interpretation of *MYBPC3* variants and can be extended to other conditions that are induced by loss of function of proteins with similar multidomain arrangements. Looking to the future, our experimental assessment suggests that extended high-resolution structural coverage of cMyBP-C can lead to better STRUM sensitivity. However, as recognized by the authors and mirroring other pathogenic mechanisms such as defects in messenger RNA (mRNA) splicing,[10] experimental validation of domain destabilization should always be pursued. This is important because it can identify rare, but definitely occurring, STRUM false positives. Indeed, current ACMG/AMP guidelines acknowledge that experimental evidence of damaging effects at the protein level provides higher evidence of pathogenicity than bioinformatics

predictions (criterion PS3 vs. PP3). We envision that combination of experimental protein stability data obtained using recombinant domains[6,8] and cell biology approaches[7] will shed light into the extent of cMyBP-C destabilization that can be tolerated by cardiomyocytes, and the cellular factors involved, refining the use of protein destabilization phenotypes in the clinical interpretation of HCM-linked genetic variants. Similar approaches should be followed in other genetic conditions caused by protein loss of function.

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REFERENCES

1. Marian Ali, J. & Braunwald, E. Hypertrophic cardiomyopathy. *Circ. Res.* **121**, 749–770 (2017).
2. Semsarian, C. & Ingles, J. in *Hypertrophic Cardiomyopathy* (ed Naidu, S. S). Genetics of HCM and role of genetic testing. (Springer-Verlag, London, 2015).
3. Thompson, A. D. et al. Computational prediction of protein subdomain stability in MYBPC3 enables clinical risk stratification in hypertrophic cardiomyopathy and enhances variant interpretation. *Genet. Med.* https://doi.org/10.1038/s41436-021-01134-9 (2021).
4. Ababou, A. et al. Myosin binding protein C positioned to play a key role in regulation of muscle contraction: structure and interactions of domain C1. *J. Mol. Biol.* **384**, 615–630 (2008).
5. Smelter, D. F., Lange, W. J. D., Cai, W., Ge, Y. & Ralphe, J. C. The HCM-linked W792R mutation in cardiac myosin-binding protein C reduces C6 FnIII domain stability. *Am J Physioll Heart Circ Physiol* **314**, H1179–H1191 (2018).
6. Pricolo, M. R. et al. Protein thermodynamic destabilization in the assessment of pathogenicity of a variant of uncertain significance in cardiac myosin binding protein C. *J. Cardiovasc. Transl. Res.* **13**, 867–877 (2020).
7. Helms Adam, S. et al. Spatial and functional distribution of MYBPC3 pathogenic variants and clinical outcomes in patients with hypertrophic cardiomyopathy. *Circ Genom Precis. Med.* **13**, 396–405 (2020).
8. Suay-Corredera, C. et al. Protein haploinsufficiency drivers identify MYBPC3 variants that cause hypertrophic cardiomyopathy. *J. Biol. Chem.* (2021). (In press).
9. Suay-Corredera, C. et al. Nanomechanical phenotypes in cardiac myosin-binding protein C mutants that cause hypertrophic cardiomyopathy. *ACS Nano* (2021). (In press).
10. Ito, K. et al. Identification of pathogenic gene mutations in LMNA and MYBPC3 that alter RNA splicing. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 7689–7694 (2017).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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