Proceedings of the 11th World Congress on Electrical Engineering and Computer Systems and Sciences (EECSS'25)
Paris, France - August, 2025
Paper No. ICBES 149
DOI: 10.11159/icbes25.149

Using Orthogonal Structural Analyses to Evaluate Variants of Unknown Significance Involved In *Mitofusin-2*

Kimiya Shafaat[1, †], Yuxin Tong[1, †], Elise Chen[1, †], Arhaan Kapoor[1, †], Sophia Liu[1, †], Joshua Pillai[1, 2,3], Kijung Sung[3], Linda Shi[1, 3], Chengbiao Wu[2, 3]

[1]Institute of Engineering in Medicine, University of California, 9500 Gilman Dr, La Jolla, San Diego, CA 92093, USA.
[2]School of Biological Sciences, University of California, 9301 S Scholars Dr, La Jolla, San Diego, CA 92093, USA.
[3]Department of Neurosciences, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0624, USA.
***** Correspondence: jppillai@ucsd.edu
†High school students participating in IEM OPALS program

Extended Abstract

Charcot-Marie-Tooth disease 2A (CMT2A) is an autosomal dominant neurological disease characterized by axonal neuropathy, and is implicated by mutations in the mitofusin-2 (*MFN2)* gene, encoding for mitofusin-2. Mitofusin-2 is a dynamin-like GTPase involved in the outer mitochondrial membrane and modulates tethering of the mitochondria to the endoplasmic reticulum. However, the biological effects of missense mutations on *MFN2* varies widely along with its phenotypes as it has been previously linked with Alzheimer's disease, Parkinson's disease, and diabetes [1]. Despite its biophysical significance, not all variants of *MFN2* have been classified as benign, gain-of-function, risk-inducing, or pathogenic, which is essential to defining their role in pathogenesis of disease. Herein, we sought to define the effects of 9 variants of unknown significance (VUS), including 4 on the GTPase domain (T236M, S249C, R280H, P251R) [2], 3 on the coiled-coil heptad-repeat (H361Y, R364P, R364W) [2], and 2 on the cytosolic heptad-repeat domain (W740S, H750S) [3], respectively. Using our previously validated protocol [4], we evaluated the functional, structural, and stability effects on VUS of mitofusin-2. We also performed our analyses on 3 pathogenic variants on Arg94 (R94Q, R94W, R94G) that have been commonly reported in patients with CMT2A as controls. Using the resolved protein structure of *MFN2* (PDB: 6JFL), structural analyses were first performed. It was found that steric hindrance (local clashes) increased among all variants except for R364P and W740S that decreased. There was no general trend observed from the mutations from the relative solvent accessibility of the replaced residue. However, it was denoted that P251R had significantly altered the structure as a charge residue was replaced and the clash scores had largely increased (+21.15%), and the R94W variant had led to damage as an expansion of the cavity was observed at 70.632 $Å^3$, respectively. Next, we evaluated the predicted pathogenicity of the VUS and found that almost all variants were labelled as pathogenic, except for discrepancies with the T236M and R364W as benign from AlphaMissense. Similarly, PolyPhen-2 had identified the W740S and H750P variants as benign. Next, we performed stability analysis, modified our protocol to include DDGEmb [5] instead of INPS-MD and DeepDDG. From all VUS, it was found that S249C significantly destabilized the structure by -0.6330 ± 0.3599 kcal/mol (p = 0.0171), R280H by -0.9554 ± 0.6885 kcal/mol (p = 0.0361), and P251R by -1.0747 ± 0.5580 kcal/mol (p = 0.0126). All remaining variants did not lead to significant stabilizing or destabilizing effects on mitofusin-2. Overall, from the orthogonal structural analysis performed in this study, we provide mechanistic insights to the critical VUS of mitofusin-2 that may be informative to clinical efforts in the near future.

Keywords: Charcot-Marie-Tooth disease 2A (CMT2A), Mitofusin-2 (MFN2), Variants of Unknown Significance (VUS), Protein Structure and Stability Analysis, Pathogenicity Prediction

References

[1] Filadi, R., Pendin, D., Pizzo, P.: "Mitofusin 2: from functions to disease" *Cell Death and Disease*, 2018, 9, (3).
[2] Abati, E., Manini, A., Velardo, D., Del Bo, R., Napoli, L., Rizzo, F., Moggio, M., Bresolin, N., Bellone, E., Bassi, M.T., D'Angelo, M.G., Comi, G.P., Corti, S.: "Clinical and genetic features of a cohort of patients with MFN2-related neuropathy" *Scientific Reports*, 2022, 12, (1).

[3] Feely, S.M.E., Laura, M., Siskind, C.E., Sottile, S., Davis, M., Gibbons, V.S., Reilly, M.M., Shy, M.E.: "MFN2 mutations cause severe phenotypes in most patients with CMT2A" *Neurology*, 2011, 76, (20), pp. 1690–1696.
[4] Pillai, J., Sung, K., Wu, C.: "Predicting the impact of missense mutations on an unresolved protein's stability, structure, and function: A case study of Alzheimer's disease-associated TREM2 R47H variant" *Computational and Structural Biotechnology Journal*, 2025, 27, pp. 564–574.
[5] Savojardo, C., Manfredi, M., Martelli, P.L., Casadio, R.: "DDGemb: predicting protein stability change upon single- and multi-point variations with embeddings and deep learning" *Bioinformatics*, 2025.