Usage Report for Fiscal Year 2020

Project Title: Protein Structure Prediction and Design

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1. Background and purpose of the project, relationship of the project with other projects

Impaired enzymatic activity in D-amino acid oxidase (DAAO) caused by missense mutations has been shown to trigger amyotrophic lateral sclerosis (ALS) through an abnormal accumulation of D-serine in the spinal cord. While loss of enzymatic functions of certain ALS-causing DAAO variants have been studied before, a detailed understanding of structure-dynamics-function relationship of the rare DAAO variants has not been investigated hitherto.

2. Specific usage status of the system and calculation method

To address this, we carried out a comprehensive study of all the reported rare DAAO variants. We mined the Project MinE Variant Browser for DAAO variants owing to its increased association with ALS. This led to the identification of 20 rare variants from different populations, among which three were previously studied. Human DAAO, a flavin-adenine-dinucleotide (FAD)-dependent oxidase, governs its neuroprotective functions via its active site residues, in addition to an active site loop comprising residues 216 to 228 that acts as an "active site lid", transitioning between a "closed" state observed in crystal structures and an "open" state required for the binding of substrate and release of product. We carried out several bioinformatics analyses in conjunction with extensive all-atom molecular dynamics (MD) simulations for wild-type (WT) and fifteen rare DAAO variants.

3. Result

We found that certain rare variants disrupted key interactions with the active site and decreased the conformational flexibility of active site loop comprising residues 216-228, which is essential for substrate binding and product release. Moreover, these variants lost crucial interactions with the cofactor flavin-adenine-dinucleotide, resulting in weaker binding affinity. A detailed inspection revealed that these variants exhibited such characteristics due to the abrogation of specific salt bridges. Taken together, our study provides a gateway into the structural-dynamic features of the rare DAAO variants and highlights the importance of informatics-based integrated analyses in the screening and prioritization of variants *a priori* to the clinical-functional characterization.

4. Conclusion

Through extensive analyses, we found that some of the variants should have a collective consequence on the active site loop, and cofactor binding of DAAO, all of which play a key role in negatively affecting the enzymatic function. This report along with previous works by us and other researchers demand that whole exome sequencing of ALS patients across diverse geographical regions is required to identify novel DAAO variants in order to develop effective therapeutic strategies. This work gives us a set of structural and dynamic attributes, which we can quickly compute and evaluate for any novel DAAO variant and predict their loss-of-function mechanisms.

5. Schedule and prospect for the future

We plan to apply our computational methodology on

other ALS relevant protein families and neurodegenerative disorders to bridge the gap between variant identification, characterization and disease manifestation through a better insight into their structure-dynamics-function relationships.

6. If no job was executed, specify the reason.

Fiscal Year 2020 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

1. Bhattacharya, S., Padhi, A. K., Junghare, V., Das, N., Ghosh, D., Roy, P., Zhang, K. Y. J., Hazra, S. (2021) Understanding the molecular interactions of inhibitors against Bla1 beta-lactamase towards unraveling the mechanism of antimicrobial resistance. *Int. J. Biol. Macromol.*, https://doi.org/10.1016/j.ijbiomac.2021.02.069.
2. Padhi, A. K., Zhang, K. Y. J. (2020) Mechanistic insights into the loss-of-function mechanisms of rare human D-amino acid oxidase variants implicated in amyotrophic lateral sclerosis. *Sci. Rep.*, **10**:17146. https://doi.org/10.1038/s41598-020-74048-2

[Conference Proceedings]

[Oral presentation]

[Poster presentation]

[Others (Book, Press release, etc.)]