Original Article

Comparative modelling and prediction of mutant structures in PSEN2 protein using computational tools

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Abstract

Aim. This study aims to model the mutant structures of PSEN2 protein associated with Alzheimer's disease by comparative modeling using computational tools. **Materials and Methods**.In the present study, we have retrieved the sequence of the protein from UniProt and a list of mutations that were predicted to have deleterious effects on PSEN2 protein from Alzforum database and performed modeling of the mutant structures using Phyre 2 , Swiss Model and trRosetta **Results.**Ten mutant structures were modeled by Comparative modeling, threading and *ab-initio* methods using three web servers.**Conclusion.**Thus, we have modeled the mutant structures by inducing each of these mutations G212V, I149T, L143H, L238P , P436L, R163C, R284G, S175C, S175F and T430M in the PSEN2 protein using three different molecular modeling web tools used in our study. These mutant structures can further be focused upon for characterization and molecular docking studies in order to find a potential cure for EOAD.

Keywords: Alzheimer's disease, EOAD, PSEN2, Novel Mutations, mutant structures, Comparative modeling, Threading, *Ab-initio*, Phyre2, SwissModel, trROSETTA.

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INTRODUCTION

Alzheimer's disease is a neurodegenerative disorder that is characterized as early-onset AD (EOAD) and late-onset AD (LOAD) (Rosenberg & Pascual, 2014). The pathogenic mutations in 3 genes, amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) have been linked with the etiology of autosomal dominant form of EOAD. (An et al., 2015). The function of a protein at a molecular level can be deciphered through the 3D structures of proteins. It provides crucial information and involves a broad spectrum of applications in life science research associated with studying any molecular mechanisms. Protein complexes are the key to understanding many cellular processes (Fuller et al., 2009). PSEN2 gene codes for Presenilin-2 protein, a 448-amino acid polypeptide(Levy-Lahad et al., 1996). Presenilin 2 protein is an integral transmembrane which is processed by proteolytic cleavage. PSEN2 is an unstable holoprotein, forms a stable and biologically active heterodimer by undergoing autocatalytic endoproteolysis within the large cytoplasmic loop domain (An et al., 2015).

In the past 5 years, around 200 research and review articles about Alzheimer's disease were published in PubMed and these studies involved deciphering the role of neurotoxicants on AD (Nisa et al., 2021), identifying Novel mutations in genes associated with AD using NGS and latest sequencing methods (Klimkowicz-Mrowiec et al., 2021) and clinical studies that focus on the pathophysiological mechanisms associated with AD which revealed clinical data that can be related to the symptoms of various forms of AD.

Our institution is passionate about high quality evidence based research and has excelled in various fields (Devarajan et al., 2021; Dhanraj & Rajeshkumar, 2021; Kamath et al., 2020; Nandhini et al., 2020; Parakh et al., 2020; Perumal et al., 2021; Pham et al., 2021; Sathiyamoorthi et al., 2021; Tesfaye Jule et al., 2021; Uganya et al., 2021). The lacuna of the research studies that are being carried out in AD is that, there are no papers that have been published so far that provides insight on the structural impact of mutations on PSEN2 protein. Modeling of mutant structures by comparative modeling has to be carried out in order to gain knowledge about the impact of mutations on the PSEN2 protein that are associated with Autosomal dominant EOAD. In this study, we aim to

model the mutant structures of PSEN2 protein by adopting modelling strategies i.e Homology modelling, Threading and *Ab initio* investigate the effects of the mutations on the protein function**.**

Materials and Methods

The proposed work was carried out at the Molecular Modeling Laboratory,Department of Bioinformatics at Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences. There is no ethical approval as human samples are not involved. The sample size used for this study is 10 which are ten mutations that were found to have deleterious effects in the PSEN2 protein.

The following is the specifications of the system used for the study. We used 7th Generation Intel(R) Core(TM) i3-7100U CPU @ 2.40GHz processor and Microsoft Windows 10.0.19042 OS. The graphics specifications is Intel(R) HD Graphics Family, Intel Corporation compatible and Memory of the system is upto 8 GB with a constant power supply between 80-85% 180 W Adapter.

The data on PSEN2 gene associated with AD was collected from the Alzforum mutations database (https://www.alzforum.org/mutations) (Kinoshita & Clark, 2007) and the amino acid sequence of the PSEN2 protein was retrieved from UniProt database (https://www.uniprot.org/) (UniProt Consortium, 2021) . Ten mutations *viz* G212V (Marín-Muñoz et al., 2016), I149T (Perrone et al., 2020) , L143H (Guerreiro et al., 2010), L238P (Blauwendraat et al., 2016)0, P436L (Han et al., 2020) , R163C (Gao et al., 2019), R284G (Hsu et al., 2018), S175C (Piscopo et al., 2010), S175F (Guven et al., 2021) and T430M (Ezquerra et al., 2003) were induced into the native sequence individually and prepared as the primary input data for performing molecular modeling.

From literature, we have identified several molecular modeling tools and softwares such as SWISS-MODEL, Phyre2, I-Tasser, trRosetta and many others. These tools were scrutinized based on accuracy of predictions and feasibility to perform modeling. Phyre2 uses advanced remote homology detection methods to build 3D models and analyze the effect of amino acid variants such as nonsynonymous SNPs (nsSNPs) for a given protein sequence (Kelley et al., 2015). SWISS-MODEL is a fully automated protein homology modelling server which extrapolates the experimental information from an evolutionary related protein structure and applies it as the template to model the 3D structure of target sequence (Waterhouse et al., 2018). trRosetta (transform restrained Rosetta) is a protein structure prediction algorithm that carries out fast and accurate predictions. The protein structure is built based on direct energy minimizations with a restrained Rosetta which include inter-residue distance and orientation distributions predicted by a deep neural network. To improve accuracy further, homologous templates are included in the network prediction (Yang et al., 2020).

The three dimensional structure for the mutations of PSEN2 were modeled by performing template based prediction using SWISS-MODEL (https://swissmodel.expasy.org/interactive) (Waterhouse et al., 2018). The FASTA sequence of PSEN2 protein retrieved from the UniProt database (ID: P49810) was incorporated with each of the mutations and uploaded as the target sequence to build model for obtaining mutant structure with each and every mutation. The model was then analyzed for QMEAN Z scores and other predicted scores along with structure validation by Ramachandran Plot.

The 3D structure of the deleterious missense mutations of PSEN2 were modeled using Phyre 2 (http://www.sbg.bio.ic.ac.uk/phyre2**)(**Kelley et al., 2015)**.**The sequence with each mutation induced manually were given as input and models were generated, the confidence and coverage scores of all the models were tabulated. The sequence with induced mutations were submitted as input data at trRosetta server (https://yanglab.nankai.edu.cn/trRosetta/) (Yang et al., 2020). The models were generated with estimated TM scores which were tabulated for respective models.

Results

Homology Modeling

The mutant structures of PSEN2 protein were modeled by template-based prediction method using SWISS-MODEL, shown in Fig. 2 with Ramachandran plot for the model containing 92.35% within favored regions, shown in Fig. 3. Table 2 shows the predicted GMQE , QMEAN and MolProbity and other scores for modeled structures.

Threading - Protein fold recognition

By using Phyre2 , the mutant structures of PSEN2 protein were modeled with a confidence of 100% and the coverage was found to 67% for the modeled structures, shown in Table 1. Figure 1 depicts the model built for P436L mutation.

***Ab-initio* modeling**

The estimated TM score for models generated using trRosetta were in the range of 0.614-0.703. The lowest score was for the mutation R284G and the highest score was for the model generated for R163C and is shown in Table 3. Figure 4 depicts the model built for P436L mutation. The plots depicting the 2D information for the P436L mutant structure from trRosetta is shown in Fig. 5.

Discussion

In this study, the mutant structures of mutations on PSEN2 protein listed in alzforum that were predicted to be deleterious were modeled using three molecular modeling tools; Phyre2, SwissModel and trRosetta. Of all the models generated by these tools, trRosetta had a greater coverage of the sequence compared to Phyre 2 and SWISS-MODEL which was evident upon visualization of the models.

Advancement in sequencing technologies such as Next Generation sequencing has geared the research studies in AD by enabling identification of novel mutations associated with PSEN2 protein (Choi et al., 2012). However, the structural impact of each of these specific mutations are yet unknown (Nisa et al., 2021). Also, performing clinical studies and wet lab studies to understand the effects of these mutations is practically impossible since it involves a humongous series of experiments and studies involving huge data, ethical issues to perform studies on AD patients and so on Computational studies aid this area of research in a great way (Klimkowicz-Mrowiec et al., 2021). Virtual screening, *in-silico* predictions for effects of mutations can be performed which can further be applied to drug development studies in order to find potential lead compounds for AD (Kelley et al., 2015).

In order to achieve this, prioritization of the mutations that govern the characteristics features such as structural stability and function of PSEN2 protein is highly crucial in order to understand the pathophysiological mechanism of PSEN 2 protein in EOAD. The study was limited to modeling the mutant structure of only 10 mutations of PSEN2 protein due to low computational power and time frame of the study. In future, complete mutant structure prediction, analysis and molecular docking studies can be carried out to aid the drug development studies related to AD research.

Conclusion

In this study, we have successfully modeled the mutant structures of the following mutations viz, G212V, I149T, L143H, L238P ,P436L, R163C, R284G, S175C, S175F and T430M of PSEN2 protein using Phyre2, SWISS-MODEL and trRosetta.The mutant can further be focused upon for functional characterization in upcoming research studies.These mutant structures can be potential drug targets and serve as an aid for drug development for identifying novel therapeutics.This study also paves way for the development of new strategies for delaying the onset of AD.

Declarations:

Conflict of Interest

The authors of this paper declare no conflict of interest.

Author Contribution

Author YM was involved in data collection, data analysis, manuscript writing. Author KAS was involved in conceptualization, guidance and critical review of manuscript.

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Figures and Tables

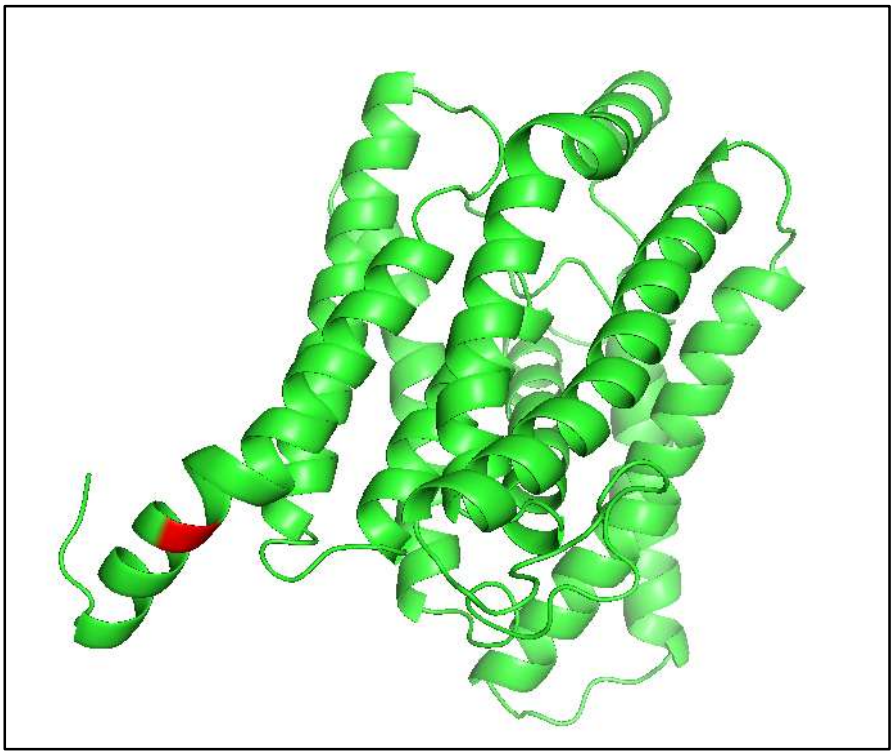


Fig. 1. 3D model of P436L mutant structure modeled using Phyre2. The red colouration indicates the mutation (P→L at 436 position).

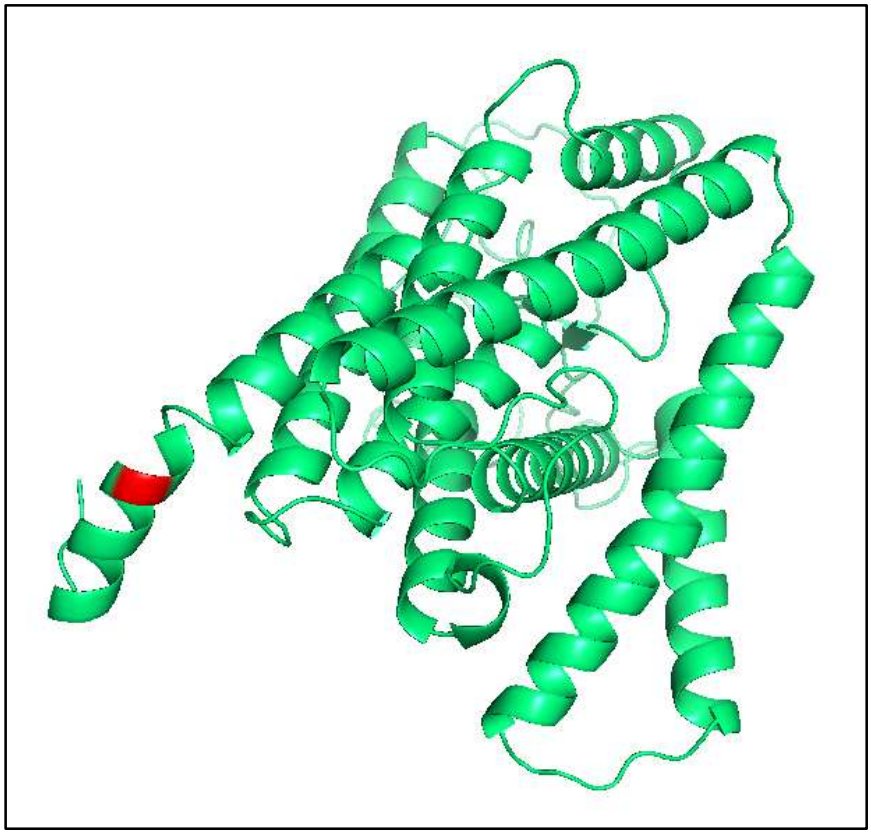


Fig. 2. 3D structure of P436L mutant in PSEN2 protein modeled by SWISS-MODEL. The mutation is distinguished by the red color in the structure.

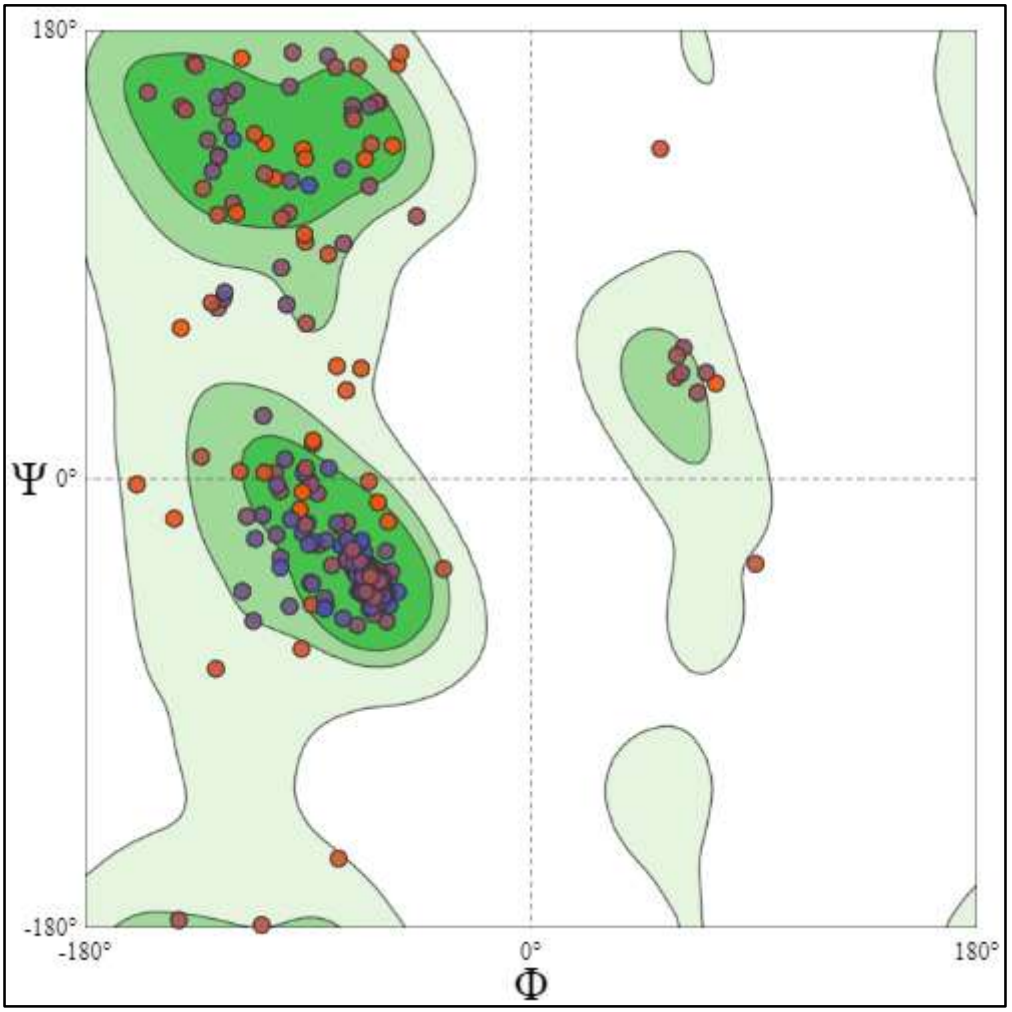


Fig. 3. Ramachandran Plot of P436L mutant structure of PSEN2 protein for the model built using SWISS-MODEL with 92.35% favoured regions.

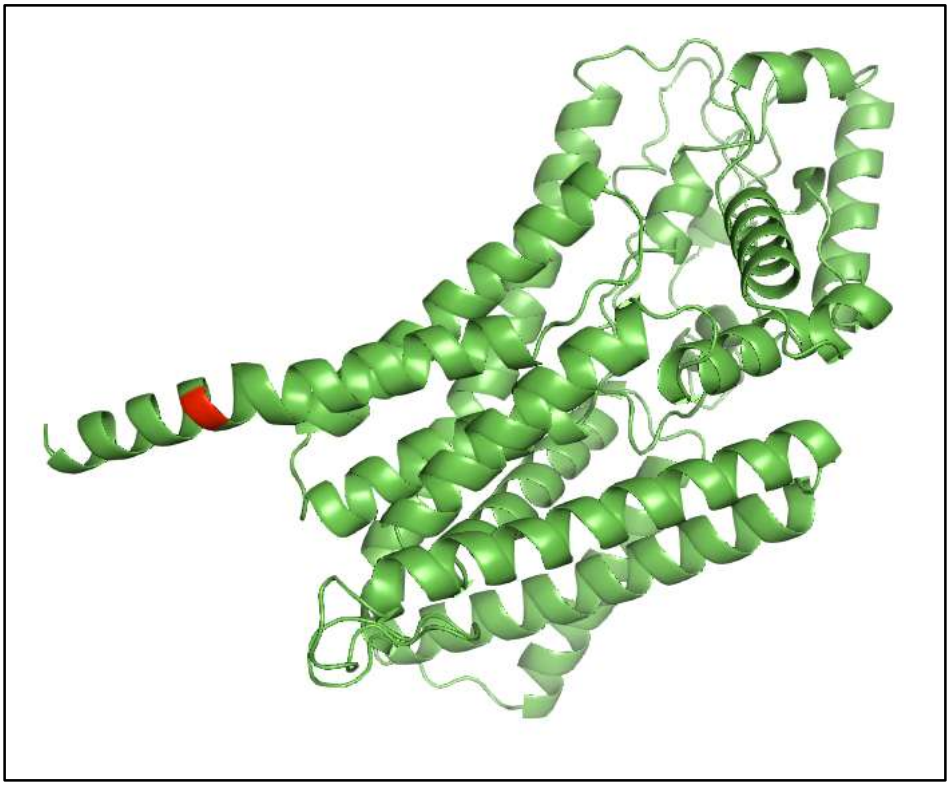
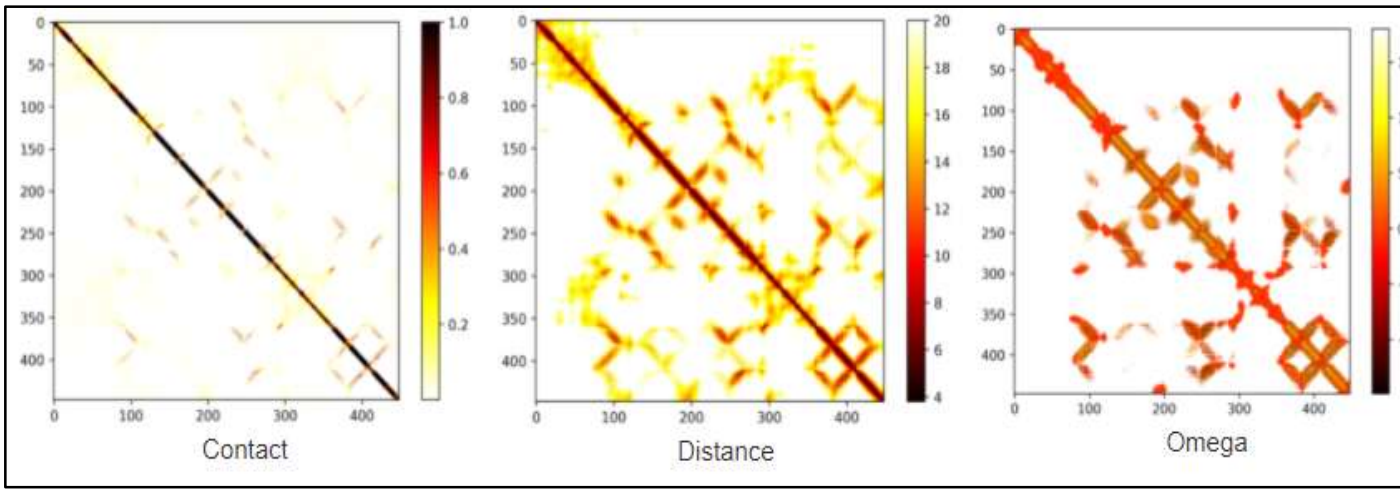


Fig. 4. 3D structure of P436L modeled using trRosetta. The site of mutation is differentiated by red colouration.



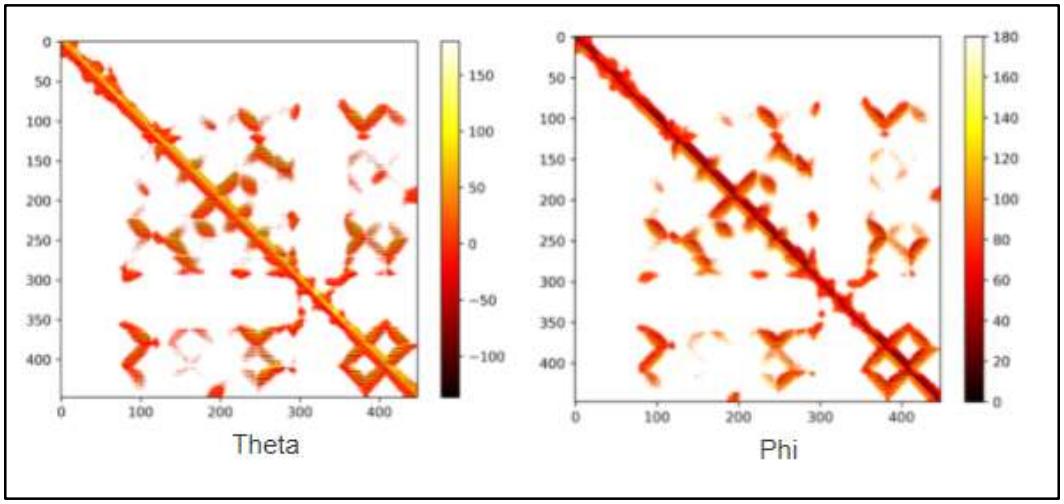


Fig. 5. Predicted 2D information about P436L mutant structure using trRosetta.

Table 1. Phyre 2 results showing Confidence and Coverage scores for the modeled structures.

Mutation	Confidence	Coverage:
G212V	100%	67%
P436L	100%	67%
I149T	100%	67%
L143H	100%	67%
L238P	100%	67%
R163C	100%	67%
R284G	100%	67%
S175F	100%	67%
T430M	100%	67%
S175C	100%	67%

Table 2. SWISS-MODEL results showing the values for various parameters for the predicted models of mutant structures.

Mutation	GMQE	QMEANDisCo Global	Template	Seq Identity	QMean	Cβ	All Atom	Solvation	Torsion	MolProbity Score	Ramachandran Favored
G212	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.54	-	0.28	0.14	-4.17	1.42	92.35%
I149	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.55	-	0.4	0.2	-4.22	1.42	92.35%
L143	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.62	-	0.34	0.2	-4.27	1.39	92.35%
L238	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.71	-	0.23	0.2	-4.37	1.4	92.35%
P436	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.54	-	0.25	0.16	-4.2	1.42	92.35%
R163	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.57	-	0.47	0.16	-4.24	1.4	92.35%
R284	0.59	0.67 ± 0.05	6lqg.1.	70.99 %	-4.87	-	0.1	0.01	-4.47	1.32	92.35%
S175	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.57	-	0.24	0.26	-4.25	1.39	92.35%
T430	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.48	-	0.36	0.3	-4.18	1.42	92.35%
S175	0.59	0.68 ± 0.05	6lqg.1.	70.99 %	-4.59	-	0.35	0.24	-4.26	1.42	92.35%

Table 3. Rosetta results for mutant models with estimated TM-score

Mutation	Estimated TM-score
G212V	0.627
I149T	0.657
L143H	0.691
L238P	0.697
P436L	0.699
R163C	0.703

R284G	0.614
S175F	0.666
T430M	0.675
S175C	0.687