In Silico Structural Modeling of Wildtype and Mutant PRODH Proteins Involved in Psychiatric Disorder.

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Abstract

Proline dehydrogenase is an important mitochondrial enzyme that is encoded by the PRODH gene. Biologically, a mutation in this gene affects the activity of proline dehydrogenase enzyme that is normally involved in conversion of proline to glutamate. However, its reduced or null activity leads to excess quantity of proline in the body, which results in different psychiatric phenotypes along with intellectual disability. In the present study, we performed in silico analysis on all reported mutations of PRODH. The 3D models of normal and mutant PRODH were predicted using I-TASSER. The predicted structures were visualized and superimposed using chimera 1.13.1. The CASTp was used to identify active sites in modelled proteins. Protein-protein docking was done with Cluspro, while protein-substrate docking was done with Auto Dock 1.5.6 and-MGL tools and the results were visualized using LigPlus+ v.2.2 and Discovery studio 2020 respectively. Alignment of 3D models (mutant with wildtype) revealed that Arg185Gln (73.83 %) and Gln199Ter (6.25 %) had the highest and lowest similarity indices, respectively. Enzyme pocket prediction identified three largest sites, with the second largest active site pocket containing substrate proline binding residues Leu527, Tyr548, and Arg563. Moreover, docking of mutant and wildtype PRODH with its close interactor “ALDH4A1” showed differences with respect to number, position, and nature of interacting amino acids residues. We observed that the nature of amino acid substitution and the number of bonds affect the binding of proline molecule with proline dehydrogenase enzyme, and therefore, affect its biological activity.

Introduction

Proline dehydrogenase, also known as proline oxidase, is a mitochondrial enzyme, encoded by PRODH gene. Proline dehydrogenase enzyme mostly found in the brain, kidney and liver. Within the cells, proline dehydrogenase has role in energy production (Campbell et al. 1997; Goodman et al. 2000). Biochemically, this enzyme is involved in catabolism of amino acid proline by first converting it to pyrrolidine-5-carboxylate. The following step converts this intermediary product to the amino acid known as glutamate (Hoogendoorn et al. 2004). The conversion between these two amino acid i.e. proline and glutamate, within the cell is very vital in keeping a supply of the amino acids required for protein production and also for the transfer of energy (Jacquet et al. 2003,2005). Decreased function of proline dehydrogenase enzyme results in the accumulation of amino acid proline in the body (hyperprolinemia) (Campbell et al. 1997) with reduced level of glutamate. And, in severe cases of hyperprolinemia, it may cause intellectual disability (ID), kidney failure, seizures, psychiatric problems and/or other neurological phenotypes (Campbell et al. 1997). Researchers believe that accumulation of proline may affect the action of different chemicals in the body that acts as neurotransmitters, and result in different psychiatric disorders such as schizophrenia (Li and He 2006; Li et al. 2004).

HGMD database has enlisted 24 missense/nonsense mutations in the PRODH gene to be involved in affecting the activity of proline dehydrogenase enzyme. Among these 24 reported mutations, 22 mutations substitute one amino acid with another amino acid (missense mutation) (Bender et al. 2005; Guilmatre et al. 2010; Jang et al. 2013), while only 2 mutations results in early truncation of the PRODH protein (non-sense mutations) (Raux et al. 2007; Kozakov et al. 2013). Most of these reported mutations compromise the efficacy of proline dehydrogenase enzyme (Jacquet et al. 2002, 2003).

The current in silico study was designed to check and compare the functional impact of all reported mutations in PRODH enzyme through protein modeling and docking, taking into account the aforementioned evidence.

Methodology

The data of all reported mutations in PRODH were obtained from HGMD database (Stenso et al. 2020), while the protein sequence was obtained from Ensembl genome browser (Ensembl genome browser 103. Ensembl.org. Accessed March 15, 2021. https://asia.ensembl.org/index.htm).

For structural analysis of normal and all PRODH mutants, 3D models were predicted using I-TASSER (Yang et al. 2015). Models with highest C-score were selected for further investigations. Visualization of 3D models were done using UCSF Chimera 1.13.1 (Pettersen et al. 2004). To investigate the differences caused by mutations, 3D models of normal and all mutant PRODH were superimposed using the Chimera.

Protein-protein docking, for normal and all mutant PRODH with their close functional interactor ALDH4A1, was done using online tool Cluspro (Kozakov et al. 2017). However, the close functional interactor of PRODH was predicted through String v9.1 database (Franceschini et al. 2013). Similarly, protein-substrate docking of normal and mutant PRODH with proline molecule was carried out through Autodock Vina and-MGL (Gaillard, 2018). The protein-substrate docked complexed were analyze through discovery studio 2020. Nonetheless, enzyme active site or binding pockets of wildtype PRODH were predicted using online tool CASTp (Binkowski et al. 2003).

Results

In general, it was observed that amino acid polarity and structure of side chain had significant impact on enzyme activity. For example, Pro406Leu and Leu441Pro, where cyclic amino acids were being replaced by aliphatic amino acids, showed severe effect. While, mild to moderate effect was observed where nonpolar but neutral amino acids were replaced by polar but uncharged amino acids and vice versa. However, this classification is very weak and cannot be implemented on all cases. The structural findings are described as follows;

Structural Analysis

The 3D models of all reported PRODH mutations (Supplementary Fig. 1) were superimposed with 3D models of wild-type PRODH protein (Fig. 1). The manual comparison of these models observed remarkable structural differences, which were measured in the form of similarity indices.

Among all the models, highest similarity index of wild-type PRODH protein with mutant was shown by Arg185Gln (73.83%), while the lowest similarity index was shown by mutant Leu441Pro + Leu441Pro/ Arg453Cys and Thr466Met + Thr466Met/Arg453Cys, which was 41.17% (Fig. 2). Complete detail of similarity
indices of all the models are summarized in Table 1.

| Mutation | Nature of Mutation | Wild-type amino acid | Substituted Amino Acid | Effect on enzyme activity | Similarity Index of mutant and normal protein | References |
|----------|--------------------|----------------------|------------------------|---------------------------|-----------------------------------------------|------------|
|          | Class | Polarity | Charge | Class | Polarity | Charge |                                   |                                              |            |
| Pro8Leu  | Cyclic | Nonpolar | Neutral | Aliphatic | Nonpolar | Neutral | Moderate | 69.33% | Jang et al. 2013 |
| Arg11Pro | Basic | Basic polar | Positive | Cyclic | Nonpolar | Neutral | Mild | 72.83% | Guilmatre et al. 2010 |
| Gln19Pro | Amide | Polar | Neutral | Cyclic | Nonpolar | Neutral | Moderate | 66.11% | Bender et al. 2005 |
| Gln19Term | Amide | Polar | Neutral | Termination occurred | | | Severe | 6.25% | Raux et al. 2007 |
| Pro30Ser | Cyclic | Nonpolar | Neutral | Hydroxylic | Polar | Neutral | Mild | 58.50% | Guilmatre et al. 2010 |
| Ala58Thr | Aliphatic | Nonpolar | Neutral | Hydroxylic | Polar | Neutral | Moderate | 68.33% | Guilmatre et al. 2010 |
| Ala167Val | Aliphatic | Nonpolar | Neutral | Aliphatic | Nonpolar | Neutral | Moderate | 69.17% | Bender et al. 2005 |
| Arg185Gln | Basic | Basic polar | Positive | Amide | Polar | Neutral | Mild | 73.83% | Bender et al. 2005 |
| Arg185Trp | Basic | Basic polar | Positive | Aromatic | Nonpolar | Neutral | Moderate | 61.33% | Bender et al. 2005 |
| Thr275Asn | Hydroxylic | Polar | Neutral | Amide | Polar | Neutral | No detrimental effect | 58.00% | Guilmatre et al. 2010 |
| Leu289Met | Aliphatic | Nonpolar | Neutral | Sulfuric | Nonpolar | Neutral | Mild | 43.17% | Bender et al. 2005 |
| Pro406Leu | Cyclic | Nonpolar | Neutral | Aliphatic | Nonpolar | Neutral | Severe | 56.83% | Bender et al. 2005 |
| Asp426Asn | Acid | Acidic polar | Negative | Amide | Polar | Neutral | Moderate | 52.00% | Bender et al. 2005 |
| Val427Met | Aliphatic | Nonpolar | Neutral | Sulfuric | Nonpolar | Neutral | Moderate | 57.67% | Bender et al. 2005 |
| Arg431His | Basic | Polar | Positive | Aromatic | Basic polar | Positive, | Moderate | 66.33% | Bender et al. 2005 |
| Leu441Pro | Aliphatic | Nonpolar | Neutral | Cyclic | Nonpolar | Neutral | Severe | 65.00% | Bender et al. 2005 |
| Gly444Asp | Aliphatic | Nonpolar | Neutral | Acid | Acidic polar | Negative, | Severe | 61.50% | Jang et al. 2013 |
| Arg453Cys | Basic | Basic polar | Positive | Sulfuric | Nonpolar | Neutral | Severe | 68.00% | Bender et al. 2005 |
| Ala455Ser | Aliphatic | Nonpolar | Neutral | Hydroxylic | Polar | Neutral | Mild | 57.83% | Bender et al. 2005 |
| Thr466Met | Hydroxylic | Polar | Neutral | Sulfuric | Nonpolar | Neutral | Severe | 51.50% | Bender et al. 2005 |
| Ala472Thr | Aliphatic | Nonpolar | Neutral | Hydroxylic | Polar | Neutral | Mild | 57.67% | Bender et al. 2005 |
| Gln521Glu | Amide | Polar | Neutral | Acid | Acidic polar | Negative, | Severe | 61.00 | Bender et al. 2005 |
| Gln521Arg | Amide | Polar | Neutral | Basic | Basic polar | Positive, | Enhance activity | 58.67% | Bender et al. 2005 |
| Gln526Term | Amide | Polar | Neutral | Termination occurred | | | Unknown effect | 61.60% | Hu et al. 2014 |
Table 2
Number of bonds and nature of amino acids docked with substrate proline molecule in all reported PRODH protein mutations

| Mutation       | Number and nature of bonding | Position and nature of bonded amino acid | H bond | Alkyl bond | C-H bond | Unfavorable bonds | Polar | Non-Polar | Basic polar | Acidic Polar |
|----------------|------------------------------|----------------------------------------|--------|------------|----------|-------------------|-------|-----------|-------------|--------------|
| Wild-type      | 2                            | Tyr548                                 | Leu527 | Arg563     |           |                   |       |           |             |              |
| Pro8Leu        | 5                            | Ala496, Val442, Leu527, Gly444         | His498, Arg443 | Glu567     |           |                   |       |           |             |              |
| Arg11Pro       | 4                            | Gly229, Phe301, Trp300                 | Arg224 | Asp228     |           |                   |       |           |             |              |
| Glu19Pro       | 2                            | Leu150                                 |        |            | Glu147, Glu556 |                   |       |           |             |              |
| Glu19Term      | Unable to dock with proline molecule due to small truncated protein | | | | | | | | | |
| Pro30Ser       | 4                            | Gln123, Tyr144, Gly552, Phe187         | Arg217 | Glu121     |           |                   |       |           |             |              |
| Ala58Thr       | 1                            | Thr411                                 | Ala381 |           |           |                   |       |           |             |              |
| Ala167Val      | 3                            | Leu527, Val442, Ala496                 | Arg564, His498, Glu567, Asp178 |           |           |                   |       |           |             |              |
| Arg185Gln      | 2                            | Ala491, Val427                         | Asp426 |           |           |                   |       |           |             |              |
| Arg185Trp      | 2                            | Ser569                                 | Val46, Pro49 | Arg324     |           |                   |       |           |             |              |
| Thr275Asn      | 3                            | Gln123, Ser210, Tyr144, Thr214         | Arg217 |           |           |                   |       |           |             |              |
| Leu289Met      | 2                            | Gln533                                 | Ala186, Ile534 | Arg579, Glu500 |           |                   |       |           |             |              |
| Pro406Leu      | 1                            | Leu561                                 |        |            | Glu60     |                   |       |           |             |              |
| Asp426Asn      | 1                            | Asn568                                 | Ala52, Ala565, Phe113 | Arg63     |           |                   |       |           |             |              |
| Val427Met      | 2                            | Tyr560, Tyr548                         | Leu527 | Arg563     | Asp380    |           |       |           |             |              |
| Arg431His      | 2                            | Asn499, Asn594                         | Leu595, Pro599, Gly574 |           |           |                   |       |           |             |              |
| Leu441Pro      | 2                            | Thr112                                 | Leu20, Phe17, Phe113 |           |           |                   |       |           |             |              |
| Gly444Asp      | 2                            | Tyr467                                 | Arg563 | Asp444, Glu567 |           |                   |       |           |             |              |
| Arg453Cys      | 5                            | Tyr551, Gln123                         | Pro553 | Arg217     | Glu121    |           |       |           |             |              |
| Ala455Ser      | 1                            | Tyr200                                 | Met555, Phe201, Trp254 | Glu418    |           |                   |       |           |             |              |
| Thr466Met      | 2                            | Asn499                                 | Val442 | His498, Arg443 | Glu567    |           |       |           |             |              |
| Ala472Thr      | 4                            | Tyr446, Tyr548                         | Leu527, Ala445 | Arg563, Lys234 | Asp380    |           |       |           |             |              |
| Gln521Glu      | 1                            | Tyr144                                 | Ala237, Leu238, Ile233 |           |           |                   |       |           |             |              |
| Gln521Arg      | 3                            | Met555, Pro559                         | Lys207 | Glu147, Glu154, Glu158 |           |                   |       |           |             |              |
| Glu526Ter      | 2                            | Arg217                                 |        |            | Asp122    |           |       |           |             |              |
| Thr466Met + Thr466Met/Arg453Cys | 1 | 580Gln | | | | | | | |
| Leu441Pro + Leu441Pro/Arg453Cys | No protein substrate bonding were noted just Van der waal forces were noted | | | | | | | | |

Active site predication.

The wild-type PRODH protein’s active site prediction revealed three major active sites. Among the three largest active sites, the second largest pocket was found to contain amino acids involved in substrate binding (proline). Leu527, Tyr548 and Arg563 are among the substrate interacting amino acids. The complete description of amino acids and its position, present in these three largest pockets, are summarized in supplementary table 1. The top three largest active site pockets of PRODH protein are illustrated in Fig. 3. It was also observed that residues in which substitution resulted in severe effect on activity of proline dehydrogenase enzyme were mostly present in the 2nd largest active site pocket. These residues include Leu441, Gly444, Arg453, Thr466 and Gln521. While, residue Pro406, exhibiting severe effect on enzyme activity, was present in the 1st largest active pocket of PRODH protein.

Protein-Protein Docking.

Protein-protein docking was carried out between wild-type and all the mutant PRODH protein with their close interactor ALDH4A1 protein and remarkable differences in the interacting sites of wild-type and mutant PRODH proteins were observed. Docking revealed that wild-type PRODH protein interacts with...
Protein-Substrate docking.

To better understand the interaction mechanism of proline, a substrate, with the wild type as well as mutant proteins, protein-substrate dockings were also performed. Wild-type PRODH was interacting with proline molecule by 3 bonds (1 hydrogen and 2 alky bond) via three residues i.e. Arg563, Tyr548 and Leu527. Highest protein-substrate interaction was shown by Pro8Leu variant, wherein the mutant protein showed interaction with proline molecule by 10 bonds (5 hydrogen, 4 alky and 1 unfavorable bond) through 7 different residues. However, the lowest protein-substrate interaction was shown by Gln19Ter variant. Wherein the mutant Gln19Ter protein was unable to interact with the proline molecule due to short shortened structure. All the interacting residues of mutant PRODH proteins with proline molecule were different as compared to wild-type. 2D representations of all the protein-substrate interaction between wild-type and mutant PRODH protein with proline molecule are in shown in Fig. 4 & supplementary Fig. 2. Similarly, compound mutant i.e. Thr466Met + Thr466Met/Arg453Cys protein was interacting with proline molecule by only 1 bond (unfavorable Donor Donor Bond) through a single residue, and compound mutant Leu441Pro + Leu441Pro/Arg453Cys protein was unable to dock with proline molecule as shown in Fig. 2.

Discussion

PRODH gene is present on chromosome 22q11.21, a region that is also reported to be associated with the contiguous gene syndrome, DiGeorge syndrome. This gene consists of 15 exons and spans 23.77 Kb of DNA. The translational product of largest transcript encodes 600 amino acids long protein (Jacquet et al. 2003). This protein acts as a proline dehydrogenase enzyme (also known as proline oxidase). Proline dehydrogenase is a mitochondrial enzyme that converts proline to Δ1-pyrroline-5-carboxylate and then to glutamate. Glutamate is the chief excitatory neuro-transmitter in the brain (Jacquet et al. 2005). PRODH mainly express in brain, lungs liver and kidney. Any pathogenic DNA change (either homozygous or compound heterozygous) in PRODH result in a condition known as hyperprolinemia type 1 (MIM#239500) and susceptibility to schizophrenia 4 (MIM# 600850). Mutation in PRODH basically affects the activity of proline dehydrogenase enzyme, which results in accumulation of proline and deficiency of glutamate in the body (Jacquet et al. 2005). This metabolic failure leads to various clinical consequences like intellectual disability (ID), kidney failure, seizures, psychiatric problems or other neurological phenotypes (Campbell et al. 1997). The investigators also found that a strain of mouse deficient in Prodh activity exhibited deficits in pre-pulse inhibition of startle, a physiological trait often impaired in patients with schizophrenia (Gogos et al. 1999).

HGMD database has enlisted 24 missense/nonsense mutations that are involved in impaired activity of proline dehydrogenase enzyme. Based on the level of reduction in enzyme activity, these mutations are divided into mild (>30% reduced enzymatic activity), moderate (>50% reduced enzymatic activity) and severe (>70% reduced enzymatic activity) mutations (Bender et al. 2005; Guilmatre et al. 2010; Jang et al. 2013). However, the investigators have also reported a missense mutation (Gln521Arg) that enhance the activity of enzyme (>120%) (Bender et al. 2005). In the current study, we tried to investigate the structural and functional impact of all reported mutations in PRODH and relate it with enzymatic activity of proline dehydrogenase. At position 521, PRODH-203 transcript had 521Arg and PRODH-215 transcript had 521Gln amino acid. So both are the natural variants of PRODH protein, while enhanced enzymatic activity due to this substitution, may be due to substitution of neutral amino acid (Gln) with positively charged amino acid (Arg). Which enhance the binding efficiency of protein with substrate proline. Q19Term mutations were severely reducing the activity of the enzyme due to short truncated premature protein. All other PRODH mutations showed variable degrees of results in 3D structures and binding to its substrate proline. The description of all reported mutations in PRODH and their documented effect on the activity of proline dehydrogenase enzyme is summarized in table (1 & 2). Our results also supported the study of Jacquet et al. (2002) that it is difficult to individually estimate the impact of PRODH mutations and their effect on the activity of proline dehydrogenase enzyme, because in most cases the individual with abnormal plasma proline levels are not simply homozygotes for a single deleterious mutation or compound heterozygotes, but may carry clusters of several protein variants, and each of them contribute collectively in the enzyme activity (Jacquet et al. 2002). As previously noted, several individuals bearing a potentially deleterious genotype had only mild hyperprolinemia with benign phenotypes because it seems unlikely that such a slight increase in proline level is sufficient to produce a detrimental effect (Hawkins et al. 2006). To confirm the finding of previous studies, we predicted the 3D models of mutation Thr466Met + Thr466Met/Arg453Cys and Leu441Pro + Leu441Pro/Arg453Cys (Hu et al. 2014; Jacquet et al. 2002) and docked them with proline molecule and found that these collective mutations further reduced the activity of PRODH protein to bind to its substrate proline as compared to all single mutated PRODH proteins. Also protein-protein interaction of mutation Thr466Met + Thr466Met/Arg453Cys with close functional interactor ALDH4A1 protein was also reduced as compared to mutation Thr466Met and Arg453Cys solely (Afenjar et al. 2007). So, we suggest that most of the time single deleterious homozygous mutation is not enough to cause a disease phenotype, it may require other heterozygous mutation and/or mutations to show their deleterious effect on enzyme activity.

Conclusion

In brief, we observed that nature of amino acid substitution and number of bonds affect the binding of proline molecule with proline dehydrogenase enzyme and hence affect its activity. In addition to the nature of mutation, we have also observed that the severity in loss of proline dehydrogenase function depends on the number of mutations that appear in a single protein, i.e. the more the number of mutation per protein the more will be the severity.

Declarations
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Ethics approval and consent to participate

The present study involved computational analysis of published mutations in PRODH and did not enrolled patients, hence exempted from ethical approval.

Competing interests

None declare by all authors

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Author's Contribution

MM, SWA & AAA performed computational data analysis and drafting of manuscript. MAK conceptualized and supervised the study and remained involved in manuscript drafting & proof read. All authors have read, edited and approved the final version of manuscript.

Data availability Statement:

The computational data is stored in the password protected personal computers of MM and MAK, which is available to editor upon request.

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Figures

Figure 1

The superimposed 3D images of wildtype PRODH protein with all reported mutant proteins
Figure 2

(a) 3D model of compound mutant (T466M+T466M/R453C) protein (b) Superimposed structure of compound mutant (T466M+T466M/R453C) with normal PRODH protein (c) 2D model of mutant (T466M+T466M/R453C) protein docked with proline molecule (d) 3D model of compound mutant (L441P+L441P/R453C) protein (e) Superimposed structure of compound mutant (L441P+L441P/R453C) with normal PRODH protein (f) Mutant (L441P+L441P+R453C) docked with proline molecule

Figure 3

Table: Pocket Analysis

| Pocket ID | Area (SA) | Volume (SA) |
|-----------|-----------|-------------|
| 1         | 3120.802  | 2479.936    |
| 2         | 510.718   | 233.510     |
| 3         | 175.343   | 61.048      |

Figure 3

Pockets on normal 3D structure of PRODH protein

3D structure of normal PRODH protein highlighting sites of all reported PRODH mutations (green)
(a) 3D structure of PRODH protein showing predicted active site pocket (b) 3D structure of normal PRODH protein highlighting site of mutation in green colour.

Figure 4

2D illustration of all protein-substrate interaction between wild-type and mutant PRODH protein with proline molecule

Supplementary Files

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