THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Characterization of Disease-Causing Mutants of Phosphoglycerate Dehydrogenase

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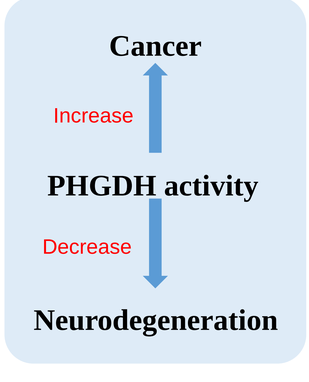
Abstract

Macular Telangiectasia type 2 (MacTel) is a rare, late-onset, macular degenerative disease associated with deficiency in circulating levels of serine. The enzyme phosphoglycerate dehydrogenase (PHGDH) is the rate-limiting enzyme in the serine synthesis pathway. It was previously identified that PHGDH is frequently mutated in patients with MacTel. Characterizing common mutations in PHGDH expressed in MacTel patients can help us understand how enzyme function relates to disease. In this study, we characterized two common loss of function mutations in MacTel patients, E297X and G228W, that resulted in a premature stop codon and a single amino acid substitution, respectively. Based on existing structural and functional data, we hypothesize that these mutations in PHGDH may impact the oligomeric structure of the enzyme. To test this hypothesis, PHGDH variants were expressed in *E. coli* BL21 cells, purified via Ni-NTA resin, and collected for further assay development. This study lays the foundation for studying the relationship between enzymatic function and oligomeric state of PHGDH and how it is impacted by loss of function mutations.

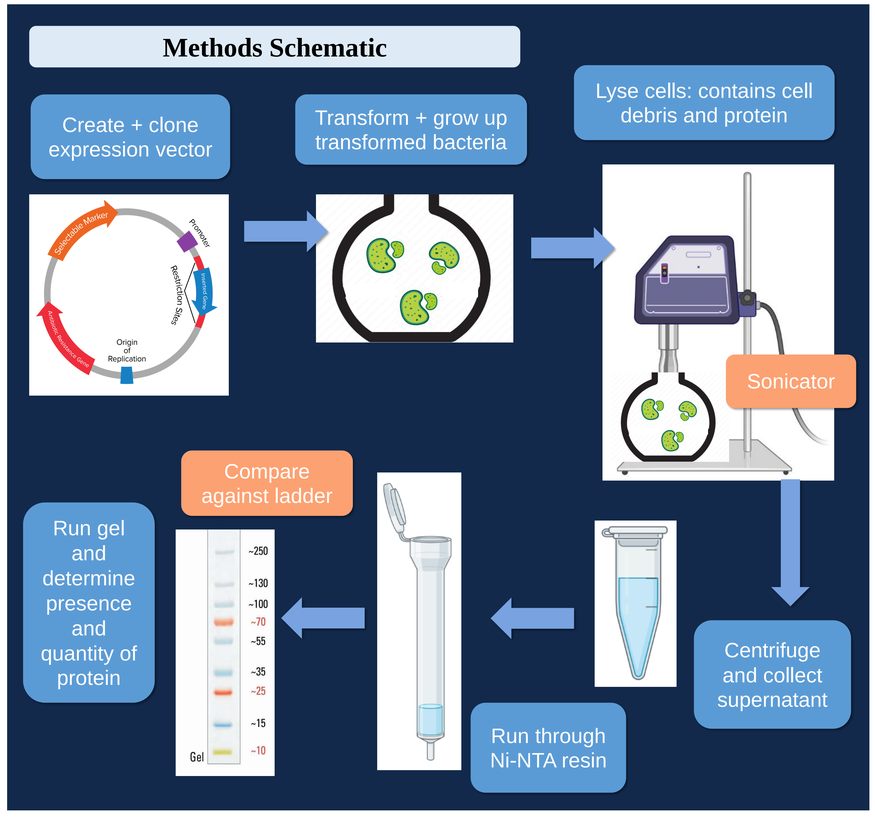
Introduction

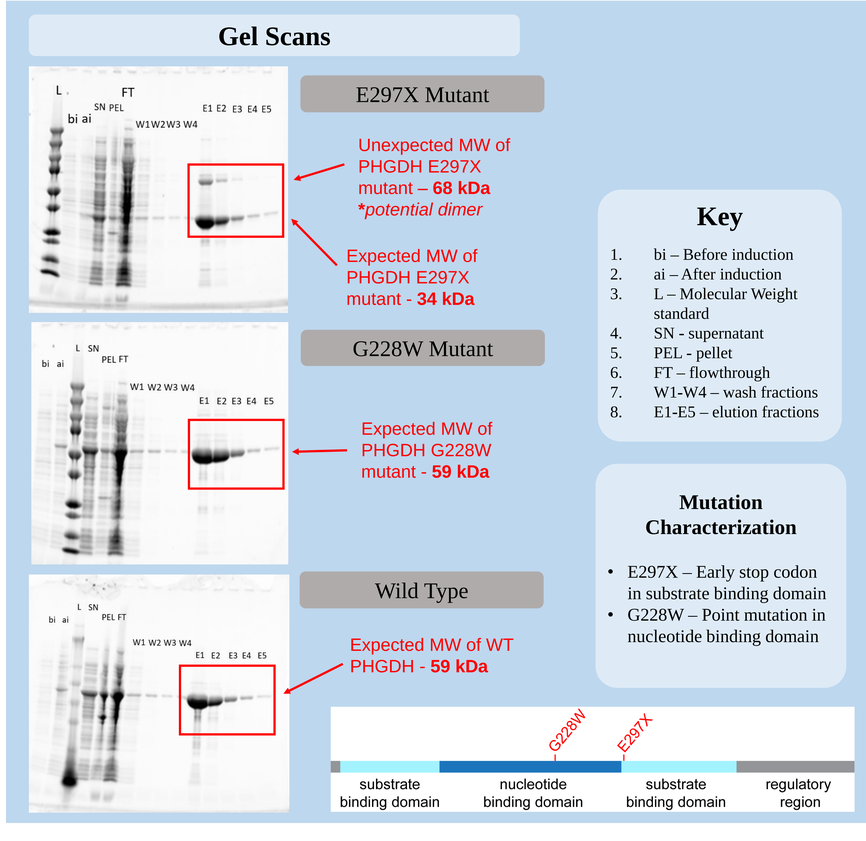
Background

- PHGDH is the rate-limiting enzyme involved in serine synthesis.
  - In cancer, activity is upregulated due to overexpression or increased availability of substrates
  - In MacTel, mutations result in a loss of enzymatic activity



- Data collected from this study can help understand how variations in PHGDH conformational states can contribute to disease
- *Hypothesis*: Mutations in PHGDH may impact the oligomeric structure of the enzyme
  - Wild type structure is hypothesized to be tetrameric, current structural data of truncated version is seen as dimeric → region missing may contribute to formation of tetrameric form

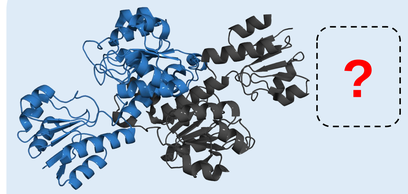




Discussion

Notable Observations/Conclusions

- PHGDH is hypothesized to have varying conformations in dimeric and tetrameric states
  - Dimeric states may be a result of cysteine interactions and formation of disulfide bonds
- The E297X mutation introduces an early stop codon
- The double band is indicative of presence of PHGDH in a dimeric state, indicating the region not translated inhibits formation of the dimer by blocking cysteine interactions



The image above highlights discrepancies in our current understanding of *normal* PHGDH conformation.

- This purification protocol is proven efficient for PHGDH as we obtained a large quantity of purified protein

Future Directions

- Continue to characterize common mutants in PHGDH
- Understand the conformational states of PHGDH in natural conditions
- Understand how varying conformational states relate to disease

References

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