Abstract 2648

Can we predict coenzyme Q deficiency in patients? The case of COQ5, a C-methyltransferase in coenzyme Q biosynthesis

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The COQ5 gene encodes an S-adenosylmethionine (AdoMet)-dependent C-methyltransferase involved in biosynthetic pathway of coenzyme Q (CoQ) in humans and in the yeast *Saccharomyces cerevisiae*. Coq5-deficient yeast are respiratory-deficient and hypersensitive to polyunsaturated fatty acid treatment, as a result of two central roles of CoQ and its reduced form (CoQH2): mitochondrial electron carrier, and chain-terminating antioxidant protecting against lipid peroxidation. Likewise, a defective CoQ biosynthetic pathway in humans can cause a wide array of illnesses, including cardiovascular, kidney, and neurodegenerative disorders, through a condition known as primary CoQ deficiency. Here, we focus on previously reported human COQ5 missense single nucleotide variants (SNVs), most of which have unknown clinical significance. We identified SNVs of potential clinical relevance using a combination of available structural, functional, and sequence information in the human COQ5 polypeptide and its close homologs. Using yeast- and *Escherichia coli*-based *in vitro* and *in vivo* studies, we plan to examine the effects of these SNVs on catalytic activity, CoQ biosynthesis, and ability to assemble or stabilize the CoQ synthome, a high molecular mass complex required for CoQ biosynthesis. These findings will be further validated through density functional theory (DFT) calculations and molecular dynamics (MD) simulations performed on the wild type and predicted mutant yeast structures, to identify differences in the energetics of catalysis and ligand binding as well as elucidate the catalytic mechanism. Our results will shed light on the structure-and-function relationship of the methyltransferase Coq5/COQ5 and its role in maintaining the CoQ synthome. By combining the power of biochemical and computational approaches, our work presents a strategy for facilitating the screening and diagnosis of primary CoQ deficiency as well as other single-gene disorders where structural information is limited.

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Abstract 2668

Characterization of novel carboxylesterase protein function using the BASIL* curriculum

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As the fundamental "workhorse" molecules of life, proteins can be found in every cell of every tissue. They appear in a vast variety of structures, each performing unique functions. Studying protein function is essential to understanding the molecular process of life along with countless biological disorders. The goal of this project was to characterize a novel protein of unknown function using computational analysis, protein purification and enzymatic activity assays. The plasmid was cultured in *E. coli* under ampicillin selection, purified and sequenced to verify identity. The BLAST alignment revealed the protein of interest to be a member of the carboxylesterase superfamily. Protein expression was induced with IPTG and harvested via B-PER® Bacterial Protein Extraction Reagent. Total protein was spectrophotometrically quantified via Bradford Protein Assay. Crude lysate proteins were resolved on 4–20% SDS-PAGE gels and results confirmed that the lysate contained a 28 kDa protein, which is consistent with the expected size of the target protein. The project is ongoing; the next steps include protein purification using immobilized metal affinity chromatography (IMAC) and functional identification via enzyme activity assay. *This project was accomplished in accordance with the protocols recommended by The Biochemistry Authentic Scientific Inquiry Laboratory Community (The BASIL Biochemistry Curriculum), basilbiochem.org, January 17, 2023. Ashley Ringer McDonald, Herbert J. Bernstein, S. Colette Daubner, Jonathan D. Dattelbaum, Anya Goodman, Bonnie L. Hall, Stefan M. Irby, Julia R. Koeppe, Jeffrey L. Mills, Stephen A. Mills, Suzanne F. O'Handley, Michael Pikaart, Rebecca Roberts, Arthur Sikora, Paul A. Craig.

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