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Structural and Functional Impact of Patient-derived Acadm Missense Mutations in Human Medium Chain Acyl-coa Dehydrogenase: Role of Fad and Rescue by Small Molecules

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Medium-chain acyl-CoA dehydrogenase (MCAD) catalyzes the first step of medium-chain fatty acids (MCFA) catabolism in mitochondrial fatty acid β-oxidation. MCAD is a homotetramer, each monomer harboring a flavin adenine dinucleotide (FAD) cofactor, incorporated during protein folding in the mitochondria. MCAD deficiency (MCADD) is a rare disease caused by mutations in the ACADM gene. No pharmacological therapy is available and dietary compliance remains an issue. Here we report the characterization of 10 recombinant MCAD variants identified in MCADD patients, combining enzymatic assays, far-UV circular dichroism, differential scanning fluorometry and limited proteolysis with molecular dynamics simulations. Despite a conserved secondary structure, the majority of the variants exhibit lower thermal and conformational stability and/or functional impairment, overall correlated with their diminished FAD binding affinity. Additionally, we observed a partial stabilizing effect of small peptides (sPep), inspired in the MCAD redox partner (electron transfer flavoprotein), towards the most common MCAD pathogenic variant (p.K329E), affording partial functional recovery. Studies are underway to test small compounds derived from sPep for further improvement. Overall, this study underlines the crucial role of FAD status in MCADD pathogenicity and offers prospective pharmacological approaches.