Recombinant ceruloplasmin to study the prevalence of Aceruloplasminemia using gnom AD.

Nicole Ziliotto[1], Sara Lencioni[1,2], Martina Cirinciani[1], Alan Zanardi[3], Massimo Alessio[3], Giulia Soldà[4], Eleonora Da Pozzo[1], Rosanna Asselta[4], Andrea Caricasole[2]

[1] Department of Pharmacy, University of Pisa, Pisa, Italy

[2] Department of Research & Innovation, Kedrion Biopharma S.p.A, Gallicano, Italy

[3] Proteome Biochemistry, COSR-Centre for Omics Sciences, IRCCS Ospedale San Raffaele, Milano, Italy

[4] Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy

ACP results from mutations in the ceruloplasmin (CP) gene, which encodes the essential ferroxidase protein found in the plasma, leading to iron accumulation in organs and multisystemic phenotypic defects related to iron metabolism. However, there is currently a limited understanding of the epidemiology of ACP.

Large-scale genomic population datasets, such as the gnomAD database, provide a valuable resource for better defining the prevalence of rare diseases, such as ACP. Nonetheless, the correct classification of missense variants as pathogenic or benign remains challenging. To address this, we devised a rational workflow incorporating the functional analysis of recombinant CP (rCP) mutants to validate in silico structural analyses for predicting the pathogenicity of the mutants, with the ultimate goal of assessing the prevalence of ACP in a real-world data population.

Essential CP residues and ACP missense mutations were extracted from the public domain. CP missense variants found in gnomAD were filtered based on this selection of CP residues. New candidate pathogenic missense variants were identified and rationally prioritized for functional characterization. Systematic biochemical and functional analyses of representative missense variants revealed varying levels of functional impairment, with some showing no significant differences from the wild-type rCP, while others demonstrated complete loss of protein function, similar to previously identified ACP mutants. This knowledge, coupled with extensive in silico structural analysis predicting the destabilizing effects of potentially pathogenic missense mutations, allowed for an estimation of ACP prevalence in the general population, including loss-of-function mutations. The occurrence of ACP in the human population appears to be higher than previously estimated, with the presence of compound heterozygotes likely accounting for much of this increased prevalence. Given the possibility of developing a protein replacement therapy for ACP, these findings can improve the diagnosis of this condition and facilitate access to future disease-modifying treatments for patients with ACP who might otherwise remain undetected.