EXPRESSION PATTERN OF PHENYLALANINE HYDROXYLASE VARIANTS IS REGULATED BY CO-CHAPERONE DNAJC12

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Background: Hyperphenylalaninemia (HPA) can be caused by deficiency in the phenylalanine hydroxylase (PAH), leading to PKU, or one of the enzymes involved in tetrahydrobiopterin (BH_4) metabolism (BH_4 deficiency). DNAJC12 deficiency has been recently described as a new form of HPA, characterized by biogenic amines deficiency, intellectual disability, movement disorder and parkinsonism. Together with the 70 kDa heat shock protein (HSP70) and nucleotide exchange factor (NEF), DNAJC12 is responsible for the proper folding of phenylalanine hydroxylase (PAH). In addition, PAH variant proteins can be refolded or degraded by DNAJC12/HSP70 machinery.

Methods: Seventeen PKU-associated PAH variants (p.F39L, p.A47V, p.R68G, p.R68S, p.E76G, p.A104D, p.D143G, p.R176L, p.V190A, p.G218V, p.R261Q, p.R297H, p.A300S, p.A313T, A403V, p.R408W, p.D415N) were transiently transfected in COS cells and cell lysates were investigated by immunoblot for DNAJC12 and PAH. Expression patterns were compared with the residual *in vitro* PAH activity.

Results: Western Blot analysis from transiently transfected PAH variants revealed endogenous expression of DNAJC12. Variants with almost no PAH protein and no PAH activity (e.g. p.R408W) showed low DNAJC12 expression, those representing typical PAH misfolding variants (e.g. p.R68G) presented with a characteristic pattern and diminished DNAJC12 expression and with 25% of PAH activity. Interestingly, some mild variants (e.g. p.A403V, p.V190A) with 30-40% residual activity showed no alterations on PAH pattern and no effect on DNAJC12 expression.

Conclusions: Our data demonstrate how DNAJC12 interacts with different PAH variant proteins. Based on this information we proposed mechanisms for refolding or degradation of PAH variants.