Determination of biochemical properties and interaction partners of human hCLPB protein - its physiological significance and role in MEGCANN syndrome pathogenesis.

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The attention of the scientific world was first brought to human CLPB protein when its malfunction was recognised to be the causative factor behind MEGCANN syndrome. The syndrome manifests itself through a variety of symptoms including 3-methylglutaconic aciduria, neutropenia, movement disorder, cataracts, as well as diverse neurological disorders ranging from nonprogressive intellectual disability to a prenatal encephalopathy with progressive brain atrophy. The most severe cases end in early death.

Very little is known about the protein itself. Its main structural features are a mitochondrial targeting sequence, ankyrin repeats and a AAA+ domain. The latter bears a strong resemblance to the second NBD domain of disaggregases present in bacteria and yeast (like ClpB from *E. coli* and Hsp104 from *S. cerevisiae*). The protein has been already reported to have an ATPase activity, but its exact function remains yet to be elucidated. What has to be noted is that despite the high similarity in the AAA+ region the protein is unlikely to be a disaggregase due to the lack of other significant features like another AAA+ module and an M domain.

The aim of the project is to characterise hCLPB in terms of its biochemistry and potential interactions. With regard to biochemistry, we intend to perform a more detailed study of the ATPase activity, optimising the reaction conditions and comparing the activity of wild type protein to that of relevant mutants present in individuals with MEGCANN syndrome. Another important aspect is the oligomer status of the protein, which, based on analogy to other AAA+ proteins, is suspected to form a hexamer. In terms of interaction partners, two groups of interactors need to be discerned, namely the substrates and cooperating proteins. While there is a number of proteins which have been shown to interact with hCLPB, none of these interactions have been studied in greater detail.

We established a procedure for overproducing our protein of interest using fermenter culture of *Pichia pastoris*. This approach proved to be superior to previous attempts using *E. coli* as it resulted in higher yield and solubility of the protein. The amount of the protein obtained allowed for establishing a preliminary purification scheme. Apart from that, we obtained constructs for expressing different variants of hCLPB corresponding to 7 mutations found in patients afflicted with MEGCANN syndrome. The mutations occur within various regions of the protein and are linked to varying severity of the symptoms present in the individuals suffering from the syndrome.