Protein replacement and gene therapy approaches in aceruloplasminemia: studies in ceruloplasmin KO mice.

Aceruloplasminemia (Acp) is a rare disease caused by mutations in the gene encoding for ceruloplasmin (CP), a ferroxidase playing a role in iron homeostasis. The absence of CP's activity leads to iron accumulation in several organs, including brain. This cause both systemic and neurodegenerative symptoms, together with the decrease of iron-restricted erythropoiesis. Due to its accumulation features, Acp displays an adult-onset and systemic symptoms may precede of about ten years the appearance of neurological symptoms. This time frame would be an important therapeutic window. Current therapy, based on iron chelation, partly controls systemic iron overload but is ineffective on neurological symptoms and is often discontinued due to side effects. Since CP is mainly expressed as circulating extracellular protein, secreted by the liver into the bloodstream and by choroid-plexus epithelial cells in the cerebrospinal fluid, we decided to explore the therapeutic potential of a protein replacement approach.

The CP-deficient mouse (cpKO) model of Acp, was weekly administered with human CP purified from plasma for 4 months in the time frame, from 6 to 10 months of age, mimicking the therapeutic window preceding neurodegeneration. Intraperitoneal-administered CP was able to reduce iron accumulation in liver, to limit steatosis and hepatic inflammation. In addition, the mobilization of iron from organs deposition promoted the rescue of erythropoiesis. Administered CP was able to enter the brain restoring the ferroxidase activity, which in turn fosters the reduction in iron deposition and neuroinflammation, the rescue of neuronal loss, and amelioration of motor coordination. CP crosses the brain barrier systems in the cpKO mice better than in wild-type animals, and this can be associated with the iron accumulation observed in the choroid plexus, which is part of the blood-cerebrospinal fluid-barrier. Indeed, cpKO-choroid plexus epithelia cells display a defect in barrier properties associated with alterations of the cell-cell adhesion structures.

To bypass the limitations of the enzyme replacement therapy, since CP is secreted in the bloodstream by the liver, we used liver-directed gene therapy aimed to establish an endogenous production of the enzyme. Two-week-old cpKO mice were transduced with liver-specific lentiviral vector carrying the human Cp gene (cpLV) and followed till 10 months of age. At the end of the treatment, more then 70% of cpLV-transduced mice exhibited circulating CP, without showing immune response anti-CP. Conversely, in the mice developing an anti-CP immune response the protein was absent. The cpLV-transduced mice showed serum ferroxidase activity with recovery of iron levels, which favored the rescue of the iron-restricted erythropoiesis. The CP produced by

cpLV-transduced mice was able to enter in the brain where it prevented iron deposition in the choroid plexus. However, the therapy was only partially efficacious in reducing the neurological symptoms, as underlined by limited rescue of both the Purkinje neurons degeneration and motor coordination performances. cpLV-transduced mice showed prevention of both liver iron deposition and steatosis, and a reduction in liver inflammation. Nevertheless, 3 out 15 cpLV-transduced mice developed a hepatocellular carcinoma independently from the CP production.

Our works indicated the potential of CP-enzyme replacement therapy in reducing both the systemic and neurological symptoms in Acp, while the liver-directed gene therapy could be a promising strategy, but further studies are mandatory to optimize the safety of the gene transduction methods.