Grant title: Molecular Insights into Primary Hyperoxaluria Type I

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Final non-technical report

Primary Hyperoxaluria Type I (PH1) is a rare genetic disease whose estimated frequency is between 1/100,000 and 1/1,000,000. The clinical symptoms of the disease are caused by the accumulation of glyoxylate that leads to the progressive deposition of insoluble calcium oxalate crystals in the kidneys and urinary tract and then, following renal failure, in the whole body. The disease is due to mutations in the gene encoding liver peroxisomal alanine:glyoxylate aminotransferase (AGT), a vitamin B6-dependent enzyme devoted to glyoxylate detoxification. AGT is present in humans as two main polymorphic variants, namely the "major" (AGT-Ma) and the "minor" (AGT-Mi) allele. The treatments currently available for the cure of PH1 are pyridoxine therapy, effective however in a minority of patients, and liver transplantation, a very invasive procedure.

PH1 is a heterogeneous disease as being caused by different pathogenic mutations with various effects at the cellular level. In spite of this heterogeneity, the understanding of the untoward consequences of PH1-causing mutations on the AGT properties at molecular level is limited. Therefore, the main objective of the funded project is the characterization of AGT in its normal and mutant forms in order to unravel the molecular defects of the pathogenic variants leading to PH1. This will constitute the basis for the development of pharmacological treatment strategies that could replace liver transplantation for patients unresponsive to pyridoxine therapy.

The results obtained during the past two years can be summarized as follows:

1) Study of the synergism between the pathogenic mutation F152I and the minor allele of AGT.

We shed light on the functional and structural consequences of the F152I mutation on the properties of both the major and the minor allelic forms of AGT. These data allowed us not only to explain why this mutation is pathogenic only when associated to the minor allele, but also to speculate about the pyridoxine responsiveness of PH1 patients bearing this mutation.

2) Investigations on the molecular bases of AGT dysfunction associated with mutations at Gly41

By means of biochemical and bioinformatic approaches, we characterized the PH1-causing AGT variants that bear mutations at Gly41 (G41R-Ma, G41R-Mi and G41V-Ma) and we drew a picture of the enzymatic phenotype of Gly41 variants more exhaustively than previously reported. In fact, on

the basis of AGT structure, the pathogenicity of these variants has been attributed to a dimerization impairment. Our results indicate that Gly41 mutation not only decreases the inter-subunit interactions, but also reduces the resistance of the protein to thermal stress and makes AGT prone to degradation and to an electrostatically-driven self-aggregation. Moreover, we reported that small osmolytes are able to reduce the aggregation extent of Gly41 variants, thus paving the way for the development of appropriated treatment strategies for PH1.

3) Analysis of the different stability under chemical stress of the major allele, the minor allele and the G170R pathogenic variant of AGT.

We compared the resistance to chemical stress of AGT-Ma, AGT-Mi and G170R-Mi both in the presence and in the absence of the coenzyme. Data obtained have shown that: i) AGT-Mi is less stable than AGT-Ma; ii) the G170R-Mi variant is indistinguishable from AGT-Mi in the presence of the coenzyme, while it shows a reduced stability of the dimeric structure in the absence of the coenzyme. These results made possible to identify the molecular defect of the G170R-Mi variant and to interpret the enzymatic phenotype of PH1 patients bearing this mutation.

Publications resulting from this project:

- B. Cellini, R. Montioli, A. Paiardini, A. Lorenzetto, C. Borri Voltattorni "Molecular insight into the synergism between the minor allele of human liver peroxisomal alanine:glyoxylate aminotransferase and the F152I mutation" **J. Biol. Chem**. (2009) 284 (13), 8349-58
- B. Cellini, R. Montioli, A. Paiardini, A. Lorenzetto, F. Maset, T. Bellini, E. Oppici, C. Borri Voltattorni "Molecular defects of the glycine 41 variants of alanine glyoxylate aminotransferase associated with primary hyperoxaluria type I" ***PNAS*** (2010) 107(7):2896-901
- B. Cellini, A. Lorenzetto, R. Montioli, E.Oppici, C. Borri Voltattorni "Human liver peroxisomal alanine:glyoxylate aminotransferase: different stability under chemical stress of the major allele, the minor allele, and its pathogenic G170R variant" **submitted for publication**
- B. Cellini, R. Montioli, A. Paiardini, A. Lorenzetto, C. Borri Voltattorni "Molecular insight into the synergism between the minor allele of human liver peroxisomal alanine:glyoxylate aminotransferase and the F152I mutation" VII European Symposium of The Protein Society, Zurich (Switzerland) June 14-18, 2009 **Poster**
- Dalibor Milic, Elisa Oppici, Barbara Cellini, Dubravka Matkovic-Calogovic "Structure of the orthorhombic crystal form of alanine:glyoxylate aminotransferase" Eighteenth Croatian-Slovenian Crystallographic Meeting, Varazdin, Croatia, June 17-21 2009, **Poster P.29**
- B. Cellini, R. Montioli, A. Paiardini, A. Lorenzetto, F. Maset, T. Bellini, E. Oppici, C. Borri Voltattorni "Molecular defects of the glycine 41 variants of alanine:glyoxylate aminotransferase associated with Primary Hyperoxaluria Type I" P-21 Meeting "Proteine 2010" Parma (Italy), April 8-10, 2010 **Oral presentation**