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GALACTOSIALIDOSIS

Galactosialidosis (GS) is a neurosomatic lysosomal storage disease belonging to the group of the glycoproteinoses. The disease is inherited as an autosomal recessive trait and is caused by genetic lesions in the *CTSA* gene encoding the lysosomal Protective Protein/Cathepsin A or PPCA. The cathepsin A enzyme's catalytic activity is distinct from its protective function towards β-galactosidase (β-GAL) and neuraminidase 1 (NEU1), with which PPCA forms a complex. In this configuration the two glycosidases acquire their full activity and stability in lysosomes. Deficiency of PPCA results in combined NEU1/β-GAL deficiency.

GS is a prototypical lysosomal storage disease (LSD) of glycoprotein catabolism and it is one of the few LSDs caused by a primary defect in one of the lysosomal cathepsins. However, the secondary severe loss of NEU1 activity probably accounts for most of the overt clinical manifestations seen in patients and for the disease pathogenesis. As for sialidosis, caused by primary deficiency of NEU1, GS primarily affects cells of the reticuloendothelial system.

Based on the age of onset and the severity of the symptoms, patients with GS are usually classified into three clinical types.

Early Infantile GS

The *early infantile type* of GS presents with signs of the disease between birth and 3-months of age; these include non-immune hydrops fetalis, edema, coarse facies, proteinuria and telangectasias. In addition, these patients develop visceromegaly, skeletal dysplasia, renal and cardiac failure and variable neurological involvement. Ocular abnormalities, including corneal clouding and cherry-red spot, and heart involvement with cardiomegaly and thickening of the septum have been seen in a number of patients. These very severe patients die within the first year of life likely because of heart and kidney failure. Importantly, early infantile type of GS may be associated with fetal loss.

Late Infantile GS

The *late infantile type* of GS comprises a distinct group of patients characterized by mild or absent cognitive impairment. Patients usually present with phenotypic alterations and symptoms within the first 2 years of life and they slowly progress into adulthood. These include coarse facies, hepatosplenomegaly, dysostosis multiplex, especially of the spine, and growth retardation associated with muscular atrophy. Heart involvement with thickening of the mitral and aortic valves, and hearing loss are recurrent features. Cherry-red spots and corneal clouding can occur in some patients, while seizures and overt neurologic signs are very rare. Many of the patients diagnosed with the late infantile phenotype survive into adulthood and are still alive. Therefore, they may develop additional kidney and pulmonary complications.

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Juvenile/Adult

The majority of GS patients belong to the *juvenile/adult type*. The reason for the dual designation is that their age of onset and clinical course vary greatly and patients present with a broad and continuous spectrum of severity of their symptoms . They are mostly of Japanese origin. In contrast to the late infantile types, patients with the juvenile/adult form of GS have a more severe clinical presentation. Besides characteristic features described above, patients develop severe neurologic manifestations, including myoclonus, cerebellar ataxia, generalized seizures, progressive cognitive impairment and mental retardation.

As it is the case for sialidosis , a few examples of atypical patients with confirmed diagnosis of GS but no oligosaccharidruria have been reported. These findings highlight the fact that GS should be taken into consideration for some of the undiagnosed cases, even in the absence of this diagnostic marker of the disease.

A total of 27 mutations in the CTSA gene have been reported, including small deletions/insertions, missense mutations, splicing variants and only one nonsense mutation.
(http://www.hgmd.cf.ac.uk/ac/index.php; http://www.ncbi.nlm.nih.gov/clinvar;
Coutinho MF et al., 2011 https://www.ncbi.nlm.nih.gov/pubmed/21214877
Caciotti et al 2013 https://www.ncbi.nlm.nih.gov/pubmed/23915561;
Annunziata and d'Azzo 2017 http://www.ncbi.nlm.nih.gov/pubmed/28603679)

Aside from its cathepsin A activity at acidic pH, PPCA also functions as a deamidase and esterase at neutral pH. In vitro, the enzyme can deamidate selected neuropeptides, such as substance P and neurokinin, and can function as carboxypeptidase on oxytocin-free acid, bradykinin, and endothelin I (ET-1). All enzymatic activities of PPCA were shown to be drastically reduced in lymphoblastoid cells and fibroblasts from numerous GS patients. In addition, it was also demonstrated that an enzyme hydrolyzing the C- terminus of ET-1 was deficient in tissues from a GS patient, a finding that implicates cathepsin A activity in the degradation of endothelin I (ET-1) in human tissues. Interestingly, increased levels of cathepsin A in heart tissue in an engineered mouse model induced the upregulation of several other cathepsins. In contrast, inhibition of cathepsin A activity has protective properties and the use of this approach has been proposed for the treatment of heart failure after myocardial infarction. More recent studies have also showed that the activities of cathepsin A and the cathepsin A homolog, serine carboxypeptidase Scpep1, are both needed to regulate ET-1 levels and to control vasoconstriction, hyper-proliferative corneal dystrophy and abnormal skin thickening. It is therefore becoming increasingly clear that lysosomal enzymes like cathepsin A can directly or indirectly been implicated in multiple biological processes that may shed light on the complexity of phenotypic alterations in patients.

(Kase et al 1990 https://www.ncbi.nlm.nih.gov/pubmed/2244901
Jackman et al 1992 https://www.ncbi.nlm.nih.gov/pubmed/1694176
Seyearantepe et al., 2008 https://www.ncbi.nlm.nih.gov/pubmed/18391110
Pan X et al., 2014 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3937211

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Pan X etal., 2017 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5325571
Annunziata and d'Azzo 2017 http://www.ncbi.nlm.nih.gov/pubmed/28603679)

PPCA and its relationship with NEU1

Extensive biochemical studies have been done on complex formation and mode of assembly of PPCA and NEU1; although a full understanding of the way these proteins interact awaits the resolution of the 3 dimensional structure of the complex. Detailed information about these studies can be found in the following papers:
(Bonten et al 2009 http://www.ncbi.nlm.nih.gov/pubmed/1966647;
Bonten et al 2014 http://www.ncbi.nlm.nih.gov/pubmed/24337808)

Mouse model of GS

A mouse homozygous for a null mutation at the *Ppca* (*Ctsa*) locus was one of the first genetically engineered animal models for an LSD. These mice have a clinical and pathological presentation that closely recapitulates the early onset forms of GS. Tissues and cells isolated from mutant mice have no cathepsin A activity and severe secondary deficiency of Neu1, while β-Gal is partially reduced only in some cell types. Extensive morphological changes are recognizable already in the first weeks of life, with severe vacuolation and lysosomal expansion in some cells of most systemic organs and the central and peripheral nervous system. As the disease primarily affects the reticuloendothelial system, pathologic changes are first detected in tissues and organs of epithelial origin. Similarly to patients with the severe form of the disease, mutant mice present with severe early-onset nephropathy, associated with edema and proteinuria, in addition to oligosaccharidurıa. Time-dependent splenomegaly and heart involvement are also characteristic features of the disease. Homozygous knockouts are infertile, because of structural changes in the blood-epididymal barrier, resulting in altered sperm motility, and have a reduced lifespan of ~6–9 months, although gender differences have been documented. The complete loss of Neu1 activity explains why many phenotypic abnormalities in Ppca KO mice are similar to those seen in the Neu1 KO model, although on close examination features that are unique for one or the other disease model have been identified. The most overt difference is seen in the cerebellum; early in life Ppca KO mice acquire acute and progressive ataxia that is associated with regional loss of cerebellar Purkinje cells and impaired motor coordination. This phenotype is not observed in the Neu1 KO mice at least not until the end of their lifespan (5–7 months). Because the expression levels of PPCA in Purkinje cells are greater than those of Neu1, it is possible that these neurons are more sensitive to the loss of cathepsin A activity than of Neu1 activity, but more rigorous testing is needed to corroborate this hypothesis.
(Zhou et al 1995 http://www.ncbi.nlm.nih.gov/pubmed/7590240;
de Geest et al 2002 http://www.ncbi.nlm.nih.gov/pubmed/12023988;
Annunziata and d'Azzo 2017 http://www.ncbi.nlm.nih.gov/pubmed/28603679)

PPCA regulates chaperone-mediated autophagy

A serendipitous finding gave the first indication of an in vivo physiological role of the cathepsin A activity of PPCA. The enzyme was found to co-purify with the lysosomal associated membrane protein 2a (LAMP2a) from lysosomal preparations of rat liver.

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LAMP2a is one of three isoforms of LAMP2 that are generated through alternative splicing of its mRNA. They are highly homologous, differing only in the composition of their transmembrane domain and short carboxy-terminal cytoplasmic tail; their heavily glycosylated, luminal domains are identical. The LAMP2a is the only isoform that serves as a receptor for chaperone-mediated autophagy (CMA). CMA is activated in response to cellular stress and promotes lysosomal internalization and degradation of cytosolic protein substrates. Human and mouse GS fibroblasts showed increased levels of CMA. Thus, Ppca-deficient cells are actively undergoing CMA.
(Cuervo et al., 2003 http://www.ncbi.nlm.nih.gov/pubmed/12505983)

Therapy for galactosialidosis

The availability of a faithful animal model for GS has been instrumental for testing investigative therapies; they include bone marrow transplantation, enzyme replacement therapy (ERT), bone marrow-mediated ex vivo and in vivo gene therapy. All these experimental approaches have been overall very successful in correcting the clinical manifestations characteristic of the disease, including partial reversal of the brain pathology. These promising results have spearheaded the development by Ultragenyx Pharmaceutical Inc. of an ERT therapy using a recombinant human PPCA (rhPPCA). The rhPPCA was s taken up by deficient human fibroblasts via the mannose-6-phosphate receptor pathway and rescued NEU1 and β-GAL activities. An in vivo proof of concept study in PPCA KO was conducted to evaluate the efficacy of rhPPCA via biweekly intravenous administration for 8 weeks. The study had as goals to evaluate the tissue distribution of rhPPCA and normalizitation of the NEU1 and β-GAL activities; to demonstrate reduction of lysosomal storage in affected tissues and to demonstrate reduction in accumulation of sialylated glycoconjugates in urine. Dose-dependent increase in cathepsin A activity in affected tissues such as liver, spleen, kidney and heart was observed; interestingly, increased cathepsin A activity was also measured in the brain, although to a smaller extent. Overall, rhPPCA treatment corrected NEU1 activity and normalized β-Gal activity in multiple tissues. In the group of mice that received the highest amount of protein (20 mg/kg rhPPCA), the vacuolization characteristic of GS was no longer evident. Finally, the treatment resulted in decreased total sialic acid in urine.
These findings should encourage the development of an ERT-mediated clinical trial for the treatment of the mild late infantile group of GS patients with no neuropathic signs.

These findings were also presented at several conferences during the last years including the ISMRD family conferences in July 2015 and November 2017.

(Zhou et al 1995 http://www.ncbi.nlm.nih.gov/pubmed/7590240;
Hahn et al 1998 https://www.ncbi.nlm.nih.gov/pubmed/9843984;
Leimig et al 2002 https://www.ncbi.nlm.nih.gov/pubmed/11964280
Bonten et al 2004 https://www.ncbi.nlm.nih.gov/pubmed/15084520
Hu et al 2012 http://www.ncbi.nlm.nih.gov/pubmed/22008912;
Annunziata and d'Azzo 2017 http://www.ncbi.nlm.nih.gov/pubmed/28603679)

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In conclusion, despite the rarity and complexity of galactosialidosis, remarkable progress has been made over the last decade towards a clinical trial for the patients. If the bench work will continue at this pace, I am inclined to predict that additional new functions for PPCA will be uncovered that might open new possibilities for alternative therapies. In this respect I believe that the most helpful hints on the roads to such discoveries often come from the patients themselves and their families. Their insightful and courageous drive is a daily encouragement for us scientists to move ahead and keep looking forward.