Title: Serum IgG glycosylation in Cystinosis.

Short Abstract:

*Lay-Oriented Description:*

We will describe a novel study on the role of antibody sugars in patients with Nephropathic Cystinosis. We will describe the methodologies involved. We will describe the extraction, purification, and glycan release technology to study the immunoglobulin G (IgG) sugars from blood serum. We will presnt the results of the study from using discrimination models and statistical approaches. This will inform on the role of IgG glycosylation (sugars) in the disease pathophysiology.

*Scientific Abstract:*

Cystinosis is a condition characterized by accumulation of the amino acid cystine within cells. Nephropathic Cystinosis, the most severe cystinosis, often manifests in infancy with failure to thrive, thirst or dehydration and is caused by mutations in the CTNS gene. We would like to explore the pathogenesis of Cystinosis. Specifically, we hypothesise that lysomal cystine accumulation initiates an inflammatory response in cystinotic cells through immunoglobulin (Ig) glycosylation dependent pathways. Some pathogenesis studies have already been undertaken to probe immune dysregulation in the context of cystinosis. One study found cystine crystals could induce proinflammatory IL-1β production through inflammasome activation (Prencipe et al, J Am Soc Nephrol., 2014, PMID: 24525029). Another study demonstrated increased titres of IgG in urine of cystinotic patients compared to healthy controls (Wilmer et al, 2008, Am J Kidney Dis., PMID: 18455850).

During immune attack, activation of the humoral immune system is initiated when antibodies recognize an antigen, bind via their Fab domains and trigger effector functions through the interaction with Fc engaging molecules termed Fc receptors (e.g. on B or NKT cells) (Vidarsson et al, 2019, Antibodies, PMID: 31544836). The most abundant immunoglobulin isotype in serum is Immunoglobulin G (IgG), which is involved in many humoral immune responses, strongly interacting with effector molecules. Studies from autoimmunity and oncology have established the role of immunoglobulin G (IgG) glycosylation, and specifically Fc glycosylation as a key modulator of immune activity (Alter et al, 2020, Glycobiology, PMID: 32103252, Novak et al, 2019, Nature Reviews, PMID: 30858582). Anecdotal evidence that Igs are playing a role in cystinosis has already been demonstrated e.g. increased titres of IgG (HMW proteins) in urine of cystinotic patients compared to healthy controls (Wilmer et al, 2008, Am J Kidney Dis., PMID: 18455850). The specific role of Ig glycosylation has never been studied in cystinosis but warrants investigations.

In this preliminary study we measured serum IgG titres and glycosylation in sera of patients with Nephropathic Cystinosis and a neurotypical cohort in a small double blinded study study (n=6 NC and n=6 neurotypical). We quantified IgG glycosylation (galactosylation, fucosylation and sialyaltion). We measured IgG glycosylation using an established methodology (O'Flaherty et al, 2019, MCP, PMID: 31471495). In short, on an automated platform we purified IgG from human serum using affinity chromatography, released the *N*-glycans with PNGase F and subsequently fluorescently labeled them with 6-aminoquinoline to visualize them by liquid chromatography. A characteristic glycoprofile was generated for each sample. The degree of glycosylation (galactosylation, fucosylation and sialylation) was quantified. Statistical analyses was performed to identify the glycosylation features that were most significantly different/clustering together in an attempt to identify the neurotypical/cystinosis cohort. Results will be presented of such findings, including unblinded results.