Bikunin, a bio-marker of genetic abnormalities that affect Golgi functions

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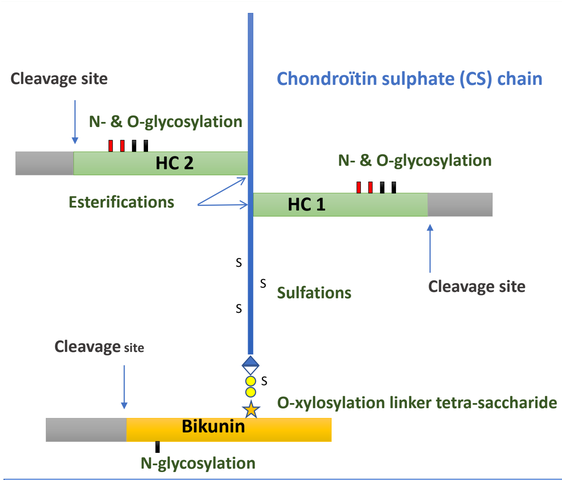
Introduction :

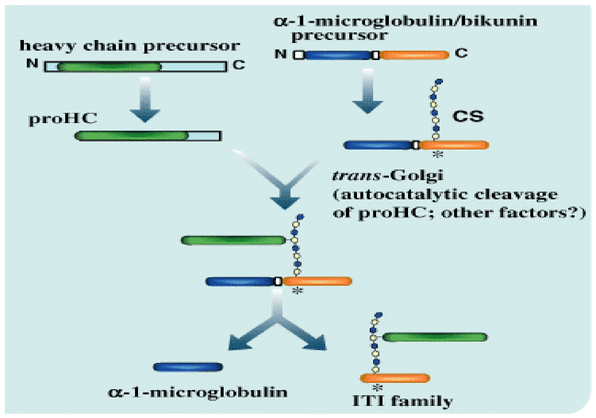
The Golgi apparatus is a dynamic intracellular organelle involved in post-translational modifications (PTM), maturation and trafficking of proteins. The organization and functions of the Golgi depend on a highly regulated environment. Thus, several molecular actors such as proton pumps, tethering factors and glycosylation enzymes are essential to ensure adequate Golgi pH, vesicular trafficking and correct post-translational modifications. Mutations in genes encoding these proteins could result in some rare diseases called "congenital disorders of glycosylation" or CDGs.

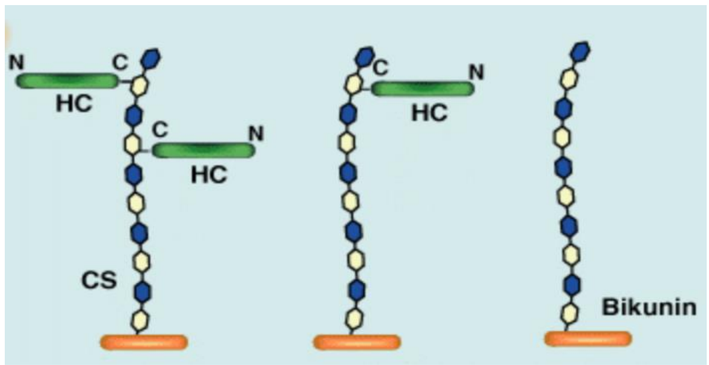
In order to improve the screening and diagnosis of some CDGs, we studied serum **bikunin** (sBkn), a chondroitin sulphate proteoglycan carrying several PTMs occurring in the Golgi. Previously, we were able to highlight, by western blotting, alterations of sBkn PTMs in patients with CDGs affecting chondroitin sulphate biosynthesis (i.e., 'linkeropathies') and in various CDGs linked to impaired Golgi homeostasis.

Our PhD project is mainly oriented towards (i) the characterization of Bkn abnormal patterns in linkeropathies (ii) the confirmation of the specificity/selectivity of this biomarker by enlarging cohorts of patients and (iii) to understand the molecular mechanisms leading to the observed alterations in both linkeropathies and CDGs.

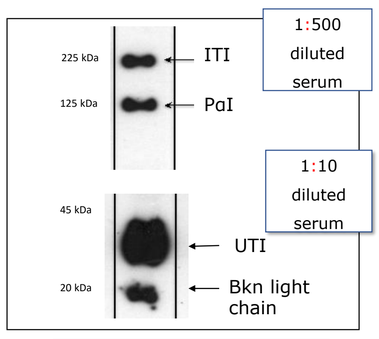
Bikunin, a serum proteoglycan that carries a large set of post-translational modifications occuring in the Golgi apparatus







ITI PaI UTI

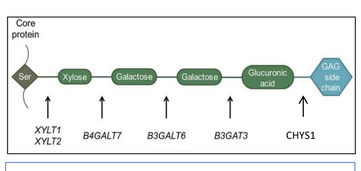


Western blot detection of serum bikunin isoforms

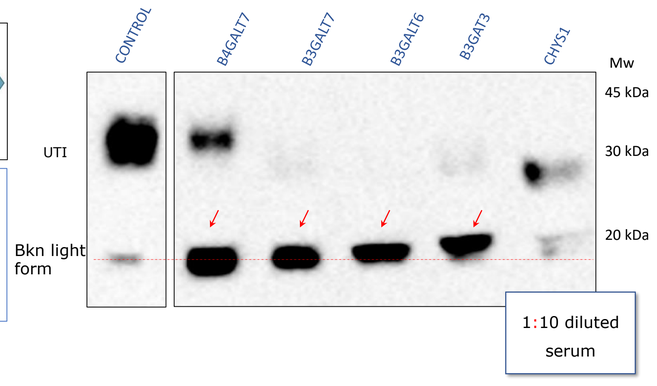
Bikunin, a circulating liver protein carrying several PTMs highly dependent on Golgi homeostasis

3 major sBkn forms : **Inter-α-trypsin inhibitor (ITI), Pre-α-trypsin inhibitor (PaI) and Urinary trypsin inhibitor (UTI).**

Achievements : Bikunin a bio-marker of linkeropathies and of CDGs related to genetic defects that affect the Golgi



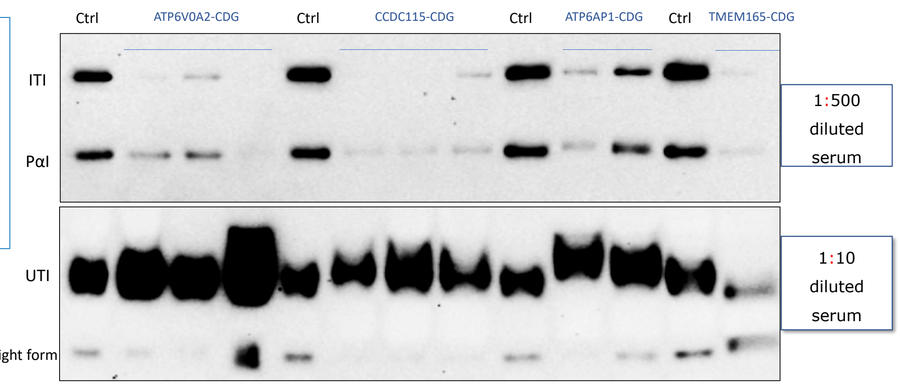
Linkeropathies:
Mutations in genes encoding for enzymes involved in the initial synthesis and elongation of *GAG*



Patients with genetic defects in B4GALT7, B3GALT6 and B3GAT3 showed **marked increase of light bikunin forms** indicating their abnormal accumulation in agreement with the stepwise action of related enzymes. Patient with altered CHYS1 showed abnormal bikunin-CS form (UTI) suggesting a defect in the CS chain elongation.
Bruneel et al. CCA 2018.

Some CDG are characterized by impairments in vesicular Golgi trafficking (COG-CDG), pH homeostasis (ATP6V0A2-CDG, CCDC115-CDG, ATP6AP1-CDG) or Mn^{2+} homeostasis (TMEM165-CDG).

Liver diseases in CCDC115-CDG, COG-CDG and ATP6AP1-CDG



➔ **(i) Reversion of the profile and (ii) overall ITI/PaI decrease** in ATP6V0A2, CCDC115 and ATP6AP1-CDG.

➔ **Significant changes of UTI and bikunin light form profile** in TMEM165-CDG.

Article in preparation.

Ongoing projects and partnerships

- Deeper characterization of abnormal forms of Bkn in linkeropathies
- Detection of sulfation defects

- 2D electrophoresis
- Enzymatic treatments (chondroïtinase, sialidase, sulfatase, phosphatase…)
- Mass spectrometry

- Enlarging the cohort of CDGs and linkeropathies patients
- **Sensitivity/specificity of Bkn**

XYLT2, SLC35C2…

SLC35A2-CDG, SLC35A3-CDG

TMEM199-CDG

TMEM165-CDG

COG-CDG

…

Patient sera, Western-blot

Clinical Data

- Understanding the molecular mechanisms leading to the observed alterations

- **Profile reversion in CDGs with impaired pH**
- **CS defects in SLC35A3-CDG**
- **UTI and bkn light chain abnormalities/decrease in TMEM165-CDG**