Bikunin proteoglycan isoforms are convenient serum biomarkers of inherited defects in glycosaminoglycan (GAG) biosynthesis and Golgi homeostasis

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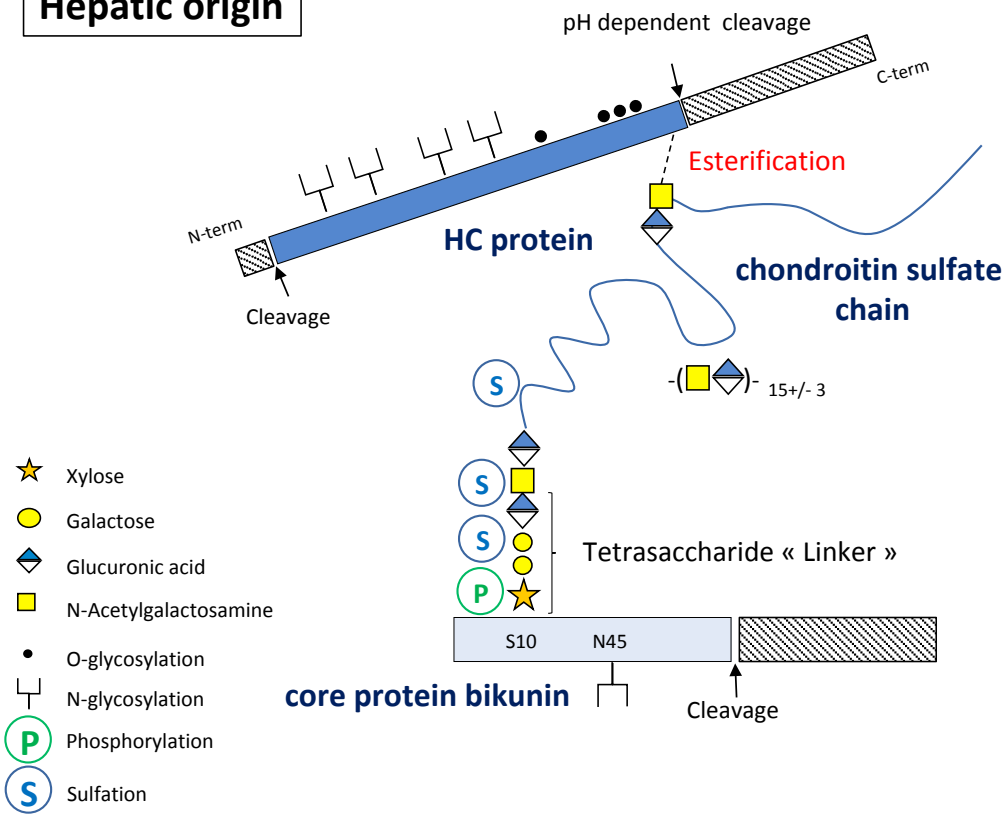
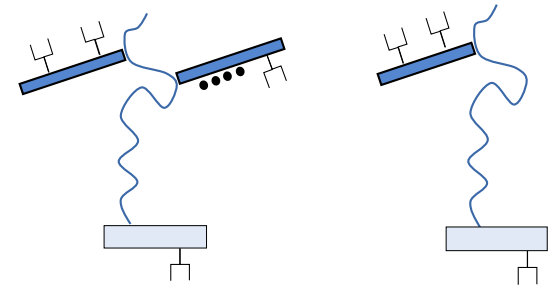
Bikunin, an original circulating proteoglycan

Hepatic origin



Golgi post-translational modifications

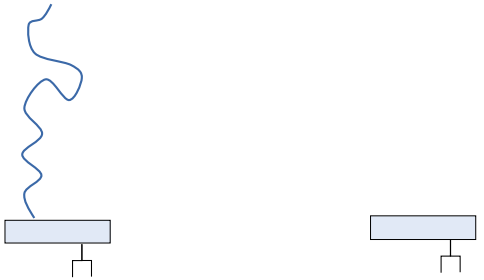
Heavy forms
90-98%



Inter-α trypsin inhibitor (ITI)

Pro-α trypsin inhibitor (PαI)

Light forms
2-10%



Urinary trypsin inhibitor (UTI)

Free core bikunin

Bikunin serum isoforms

Congenital disorders of glycosylation (CDGs)

- Inborn errors of glycan biosynthesis and branching on proteins and lipids
  - Enzymes, sugar transporters, Golgi homeostasis regulators

- Very rare diseases
  - 1300-1400 cases in Europe

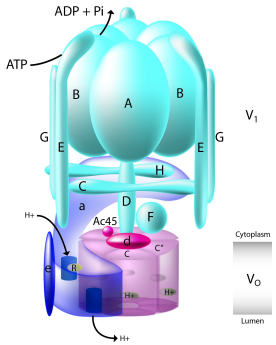
- Wide and unspecific symptomatology
  - Multiorgan alterations
  - Different clinical board for a same mutation

- Difficult biochemical/molecular diagnosis
  - Serum transferrin (N-glycosylation)
  - Serum apoC-III (mucin core O-glycosylation)
  - MS N-glycan profiling
  - Gene panel sequencing, WES

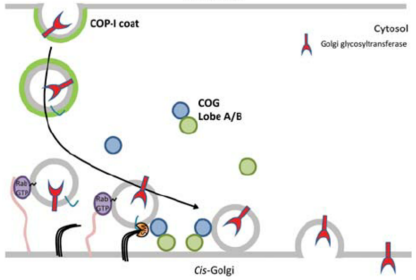
Serum bikunin
could be an additional biomarker

CDGs with impaired Golgi homeostasis

- **Golgi pH homeostasis**
  - ATP6V0A2
  - ATP6AP1
  - CCDC115



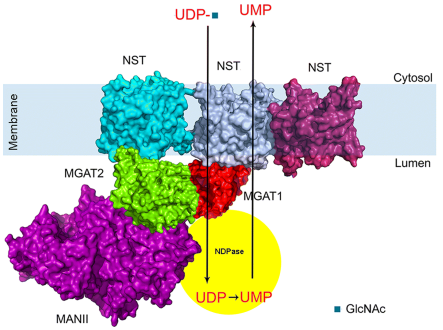
- **Vesicular tethering factors**
  - COG5
  - COG7



Reynders et al. 2010

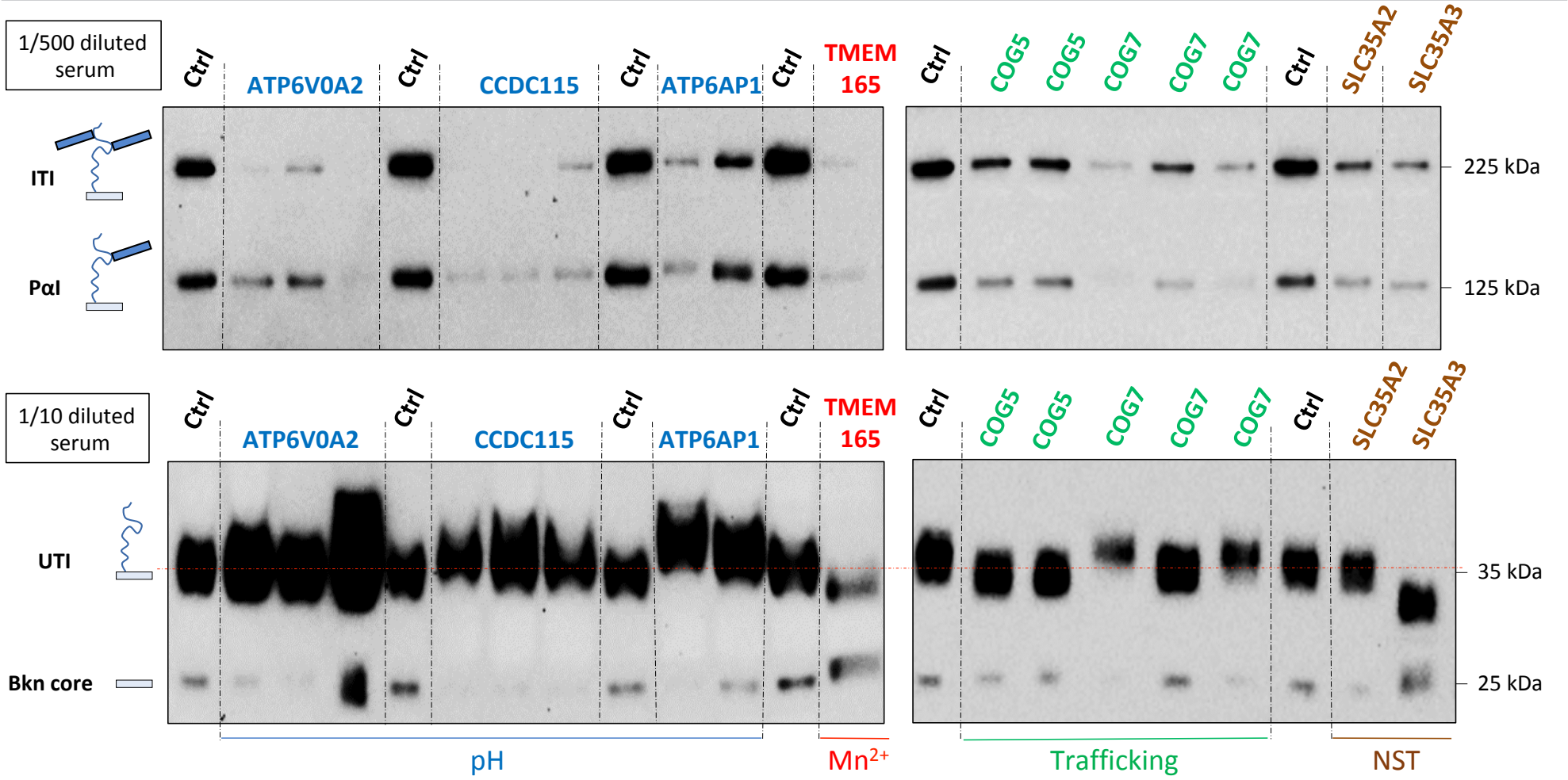
- **Golgi manganese homeostasis**
  - TMEM165

- **Golgi nucleotide sugar transporters (NST)**
  - SLC35A2 (Galactose)
  - SLC35A3 (N-acetyl glucosamine)



Khoder-Agha et al. 2019

Alterations of serum bikunin isoforms in CDG



Conclusions for serum bikunin analysis in CDGs

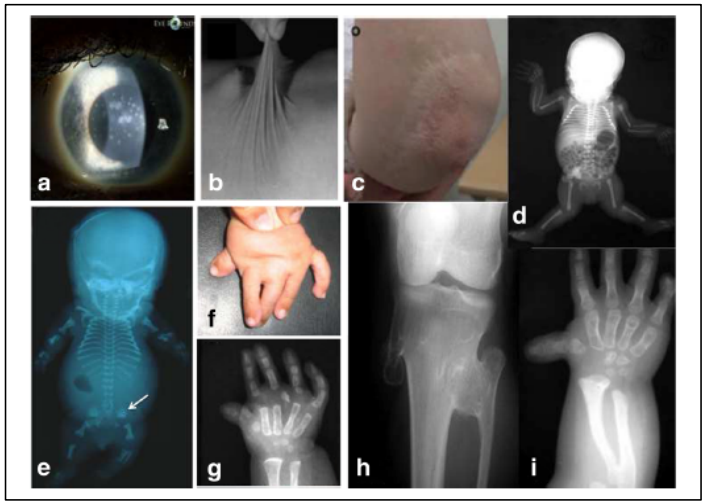
- **Alterations in the pH dependent esterification of the CS chain by HCs**
  - Mutations on Golgi H^+ V-ATPase regulators (pH homeostasis)
  - TMEM 165 (Manganese/Calcium/pH homeostasis)

- **Alterations in the CS chain elongation**
  - TMEM 165
  - SLC35A3 (GlcNAc transporter)

→ Osteoarticular phenotype of the patients

Linkeropathies

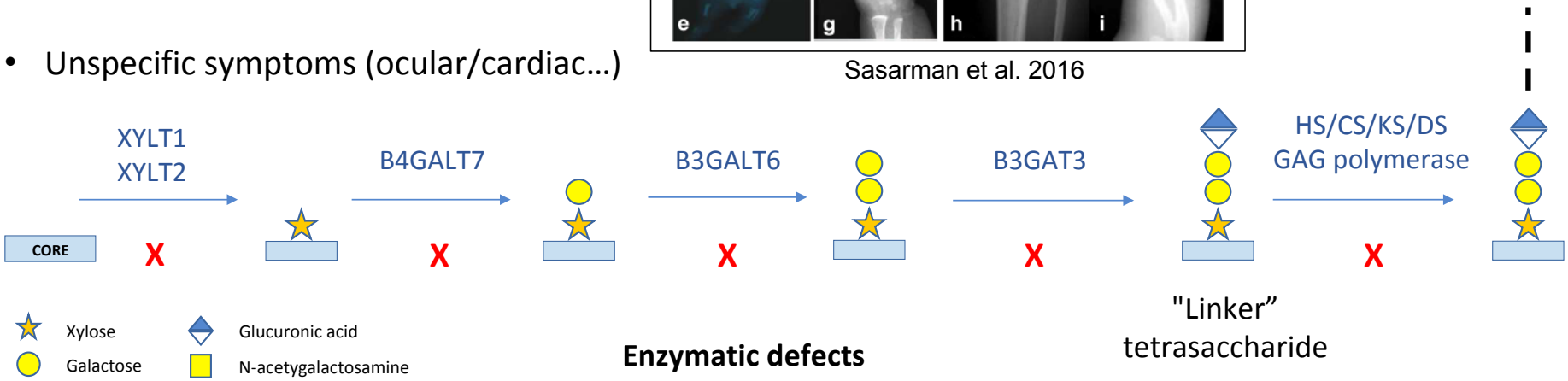
- Inborn errors of GAG biosynthesis
- Very rare diseases & under-diagnosed
  ~ 200 reported patients
- **Osteoarticular symptoms**
  (short stature/joint dislocation)
- Growth/intellectual retardation
- Unspecific symptoms (ocular/cardiac…)



Sasarman et al. 2016

"Linker" → Tetrasaccharide
Joints

Defective GAG elongation



Enzymatic defects

"Linker" tetrasaccharide

Screening of linkeropathies needs new biomarkers

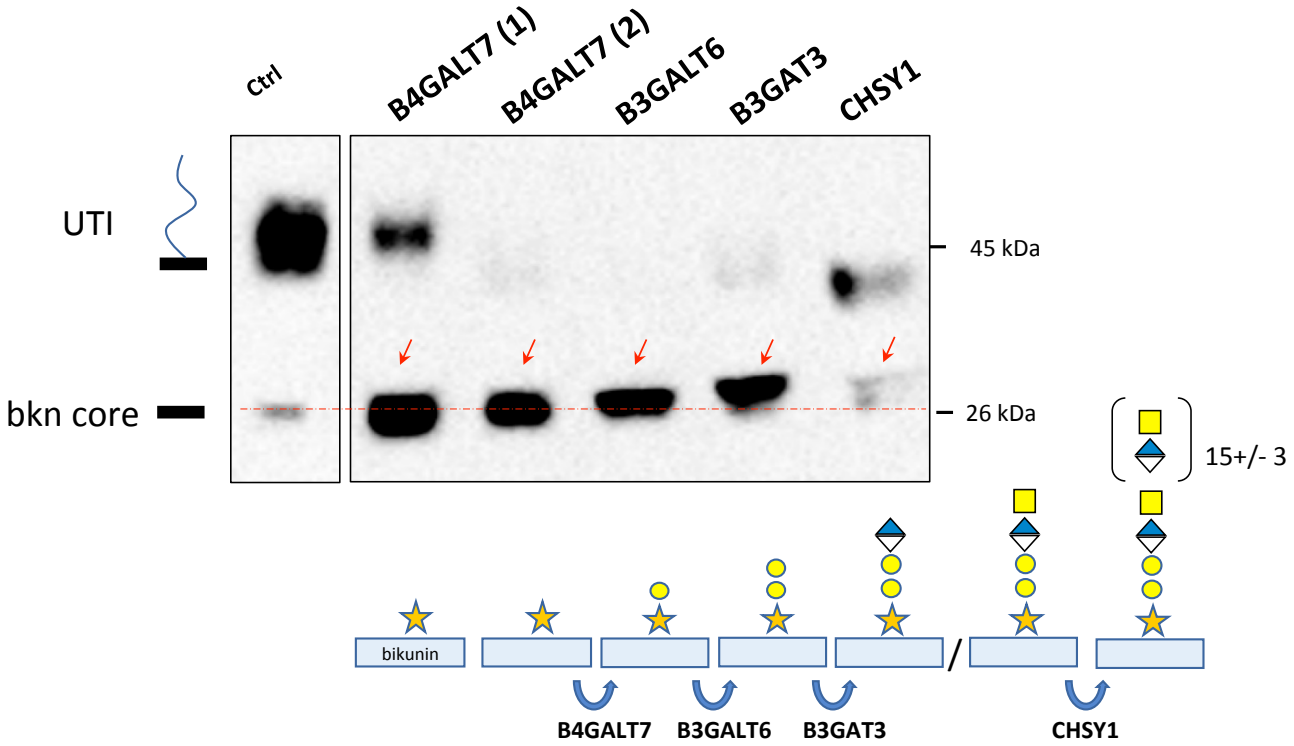
- Current biochemical screening is difficult …
  - ➢ Enzyme activity measurements (patients fibroblasts, substrates, mRNA levels)
  - ➢ GAG quantification (patient fibroblasts, ^{35}S incorporation assays, IHC)
  - ➢ Mass spectrometry after GAG purification
  - ➢ Gene panel sequencing, whole exome sequencing

Lack of blood biomarkers ⇨ Serum bikunin

- ➢ Carrying a GAG chain with the tetrasaccharide linker
- ➢ Availability in blood/serum/plasma/urine samples
- ➢ Simple and sensitive detection (antibodies)

Alteration of serum bikunin light forms in linkeropathy patients

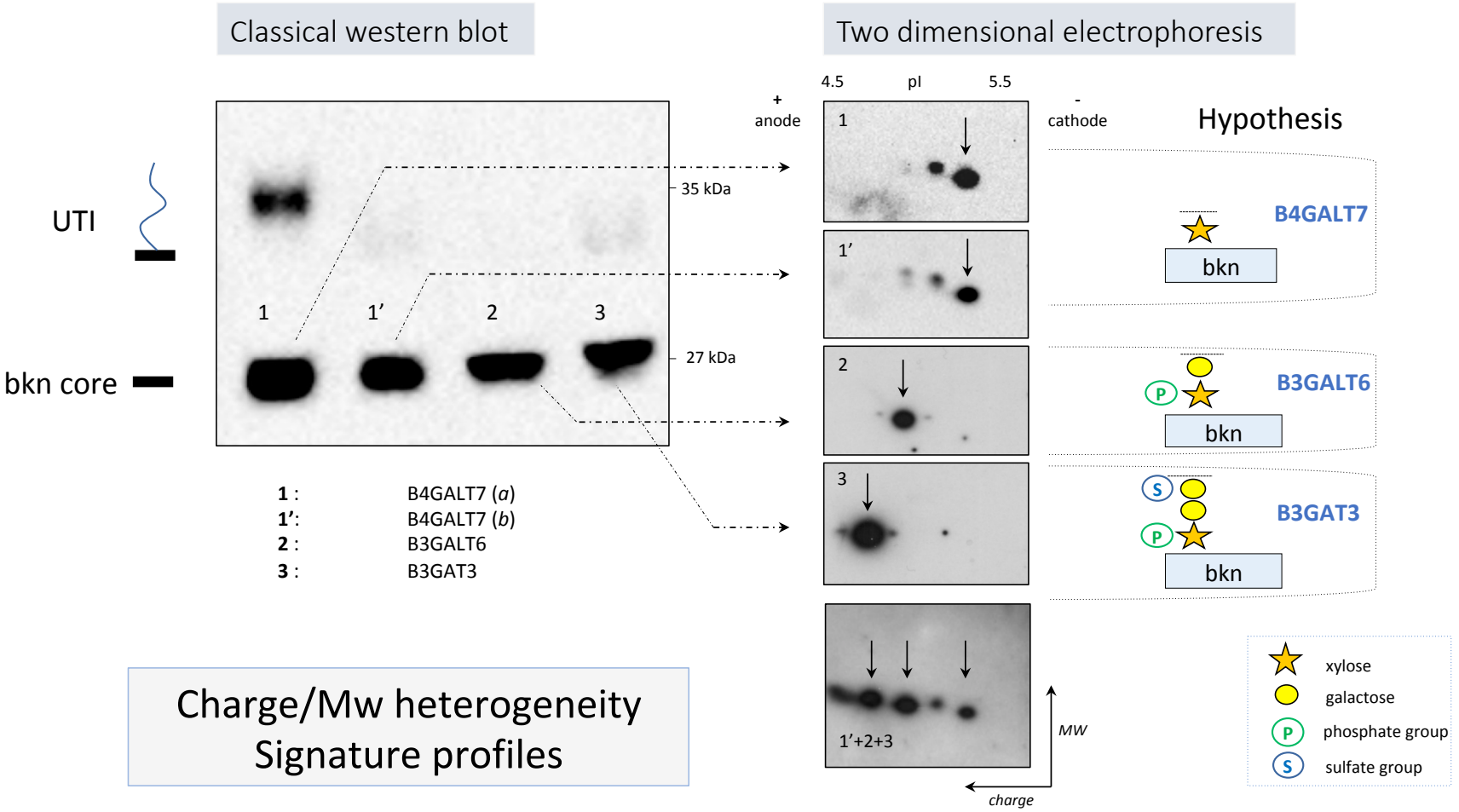
Western-blot
(anti-bikunin antibody)



Xylose
Galactose
Glucuronic acid
N-Acetylgalactosamine

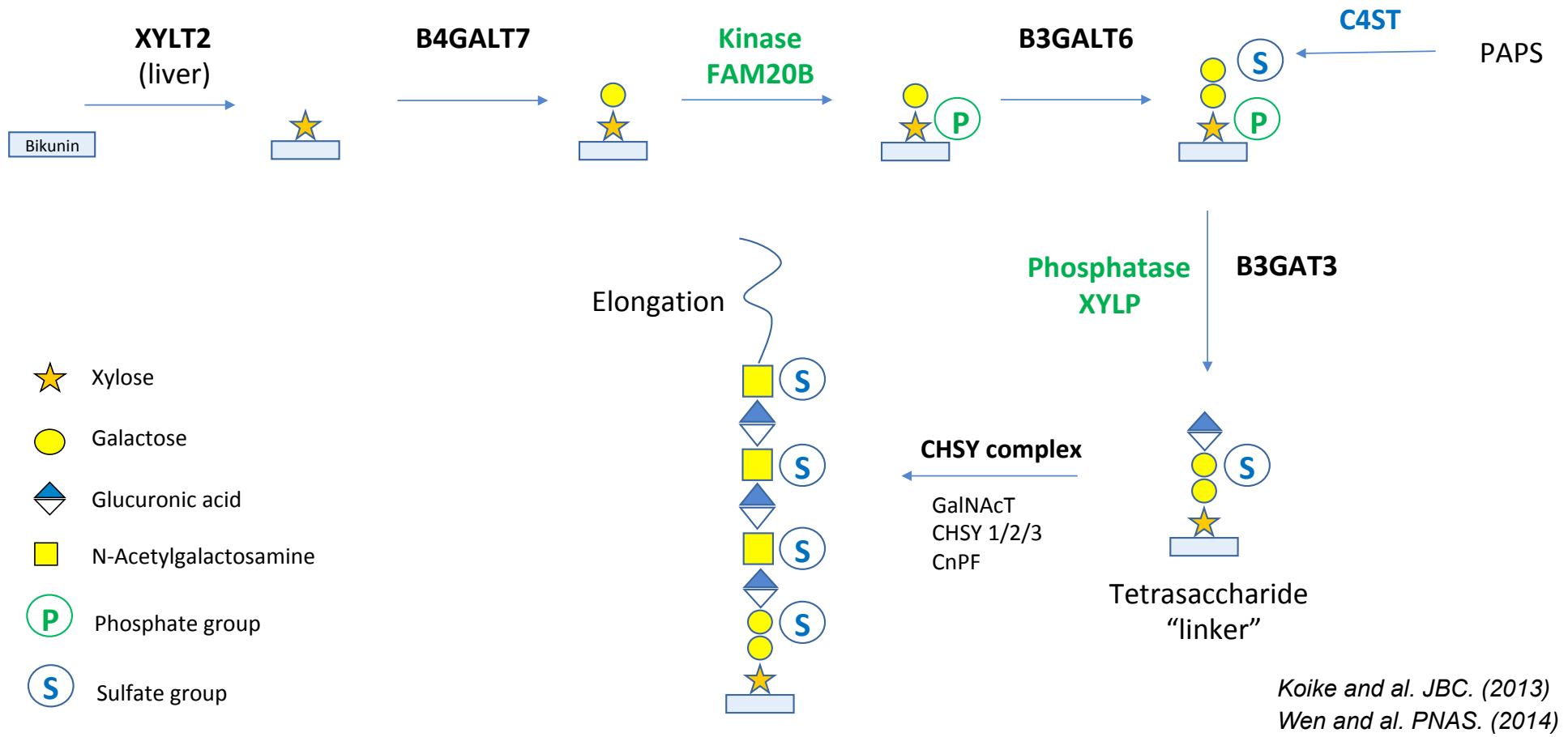
Abnormal forms with an increasing molecular weight

Characterization of bikunin abnormal forms in linkeropathies (1)



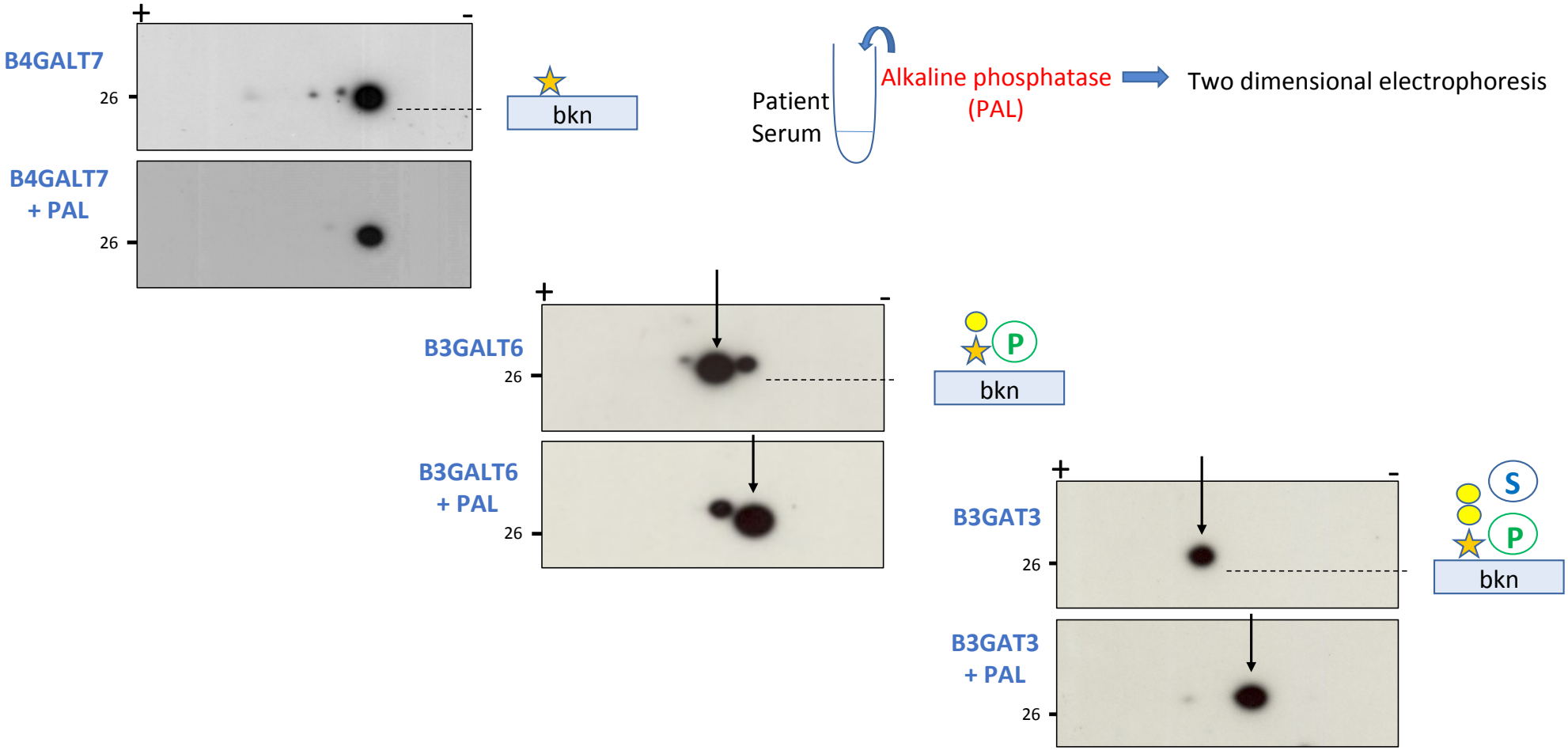
Charge/Mw heterogeneity
Signature profiles

Bikunin tetrasaccharide biosynthesis & modifications

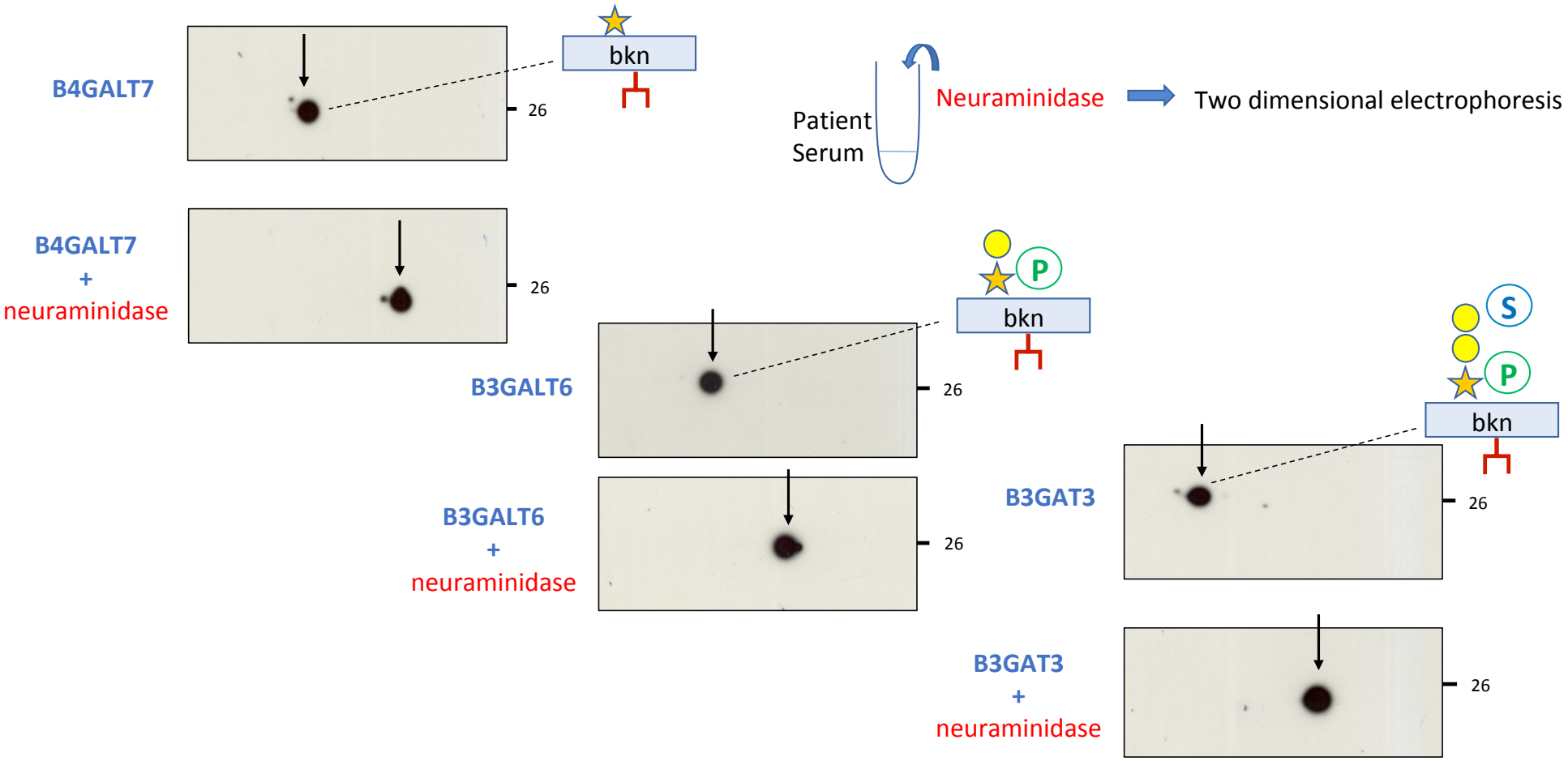


Koike and al. JBC. (2013)
Wen and al. PNAS. (2014)

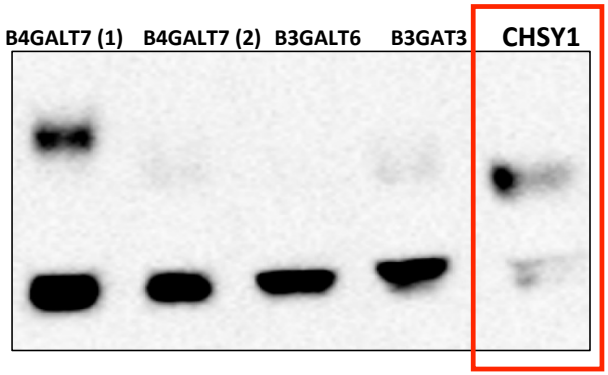
Characterization of bikunin abnormal forms in linkeropathies (2)

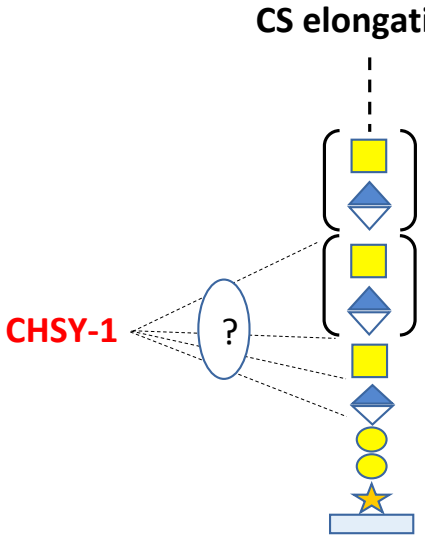


Characterization of bikunin abnormal forms in linkeropathies (3)

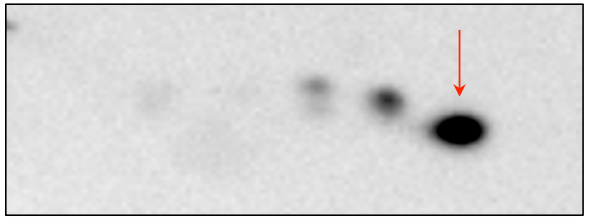


Involvement of CHSY-1 in several steps of the CS elongation



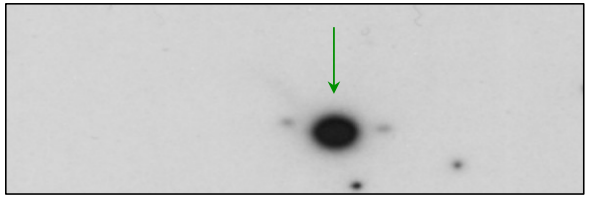


B4GALT7



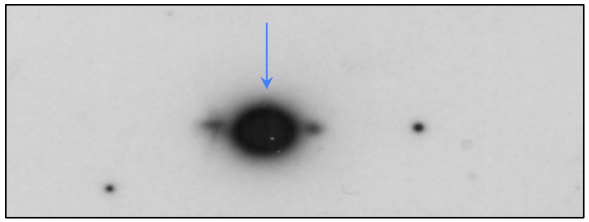
bkn

B3GALT6



bkn

B3GAT3



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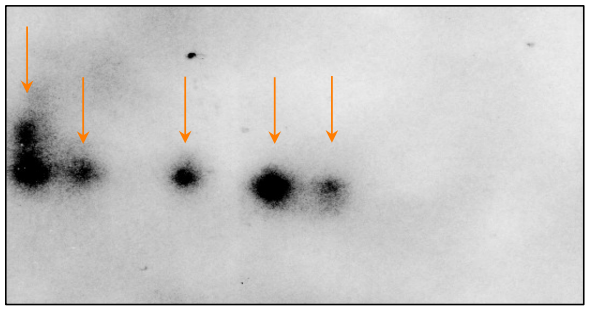
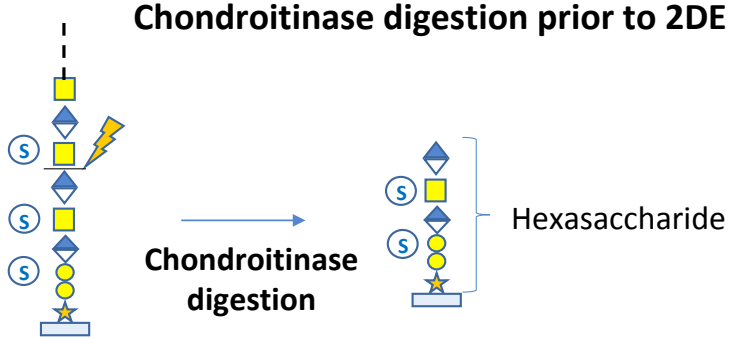
CHSY-1



heterogeneous profile

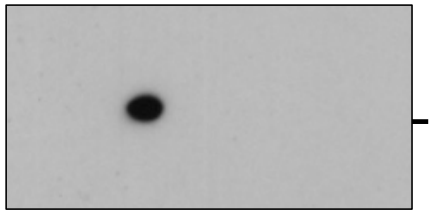
Strategy to detect sulfation defects

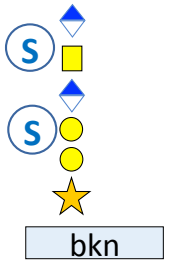
Chondroitinase digestion prior to 2DE



PAPST = activated sulfate transporter (Golgi)

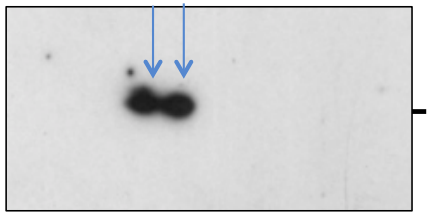
Control serum + chondroitinase

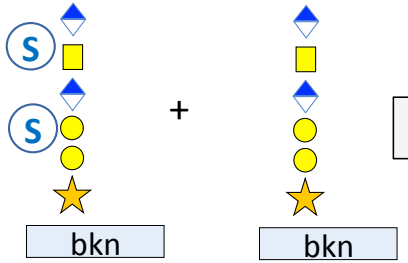




Homogeneous sulfation

PAPST + chondroitinase





Forms lacking sulfate groups

Conclusions/perspectives

Relevant structures of bikunin isoforms allow to easily screen GAG defects

Profile alterations are evidenced in some CDG with impaired Golgi homeostasis

First intention biomarker in linkeropathies
(exception: XYLT1)

Additional biomarker in CDG

Perspectives

- Abnormal forms characterization in linkeropathies by mass spectrometry
- Specificity/sensitivity in CDG by enlarging the cohort
- *In vitro/in vivo* experiments (siRNA, KO mice)

Acknowledgments

Project coordinators

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"Mécanismes cellulaires et moléculaires de l'adaptation au stress et cancérogenèse"

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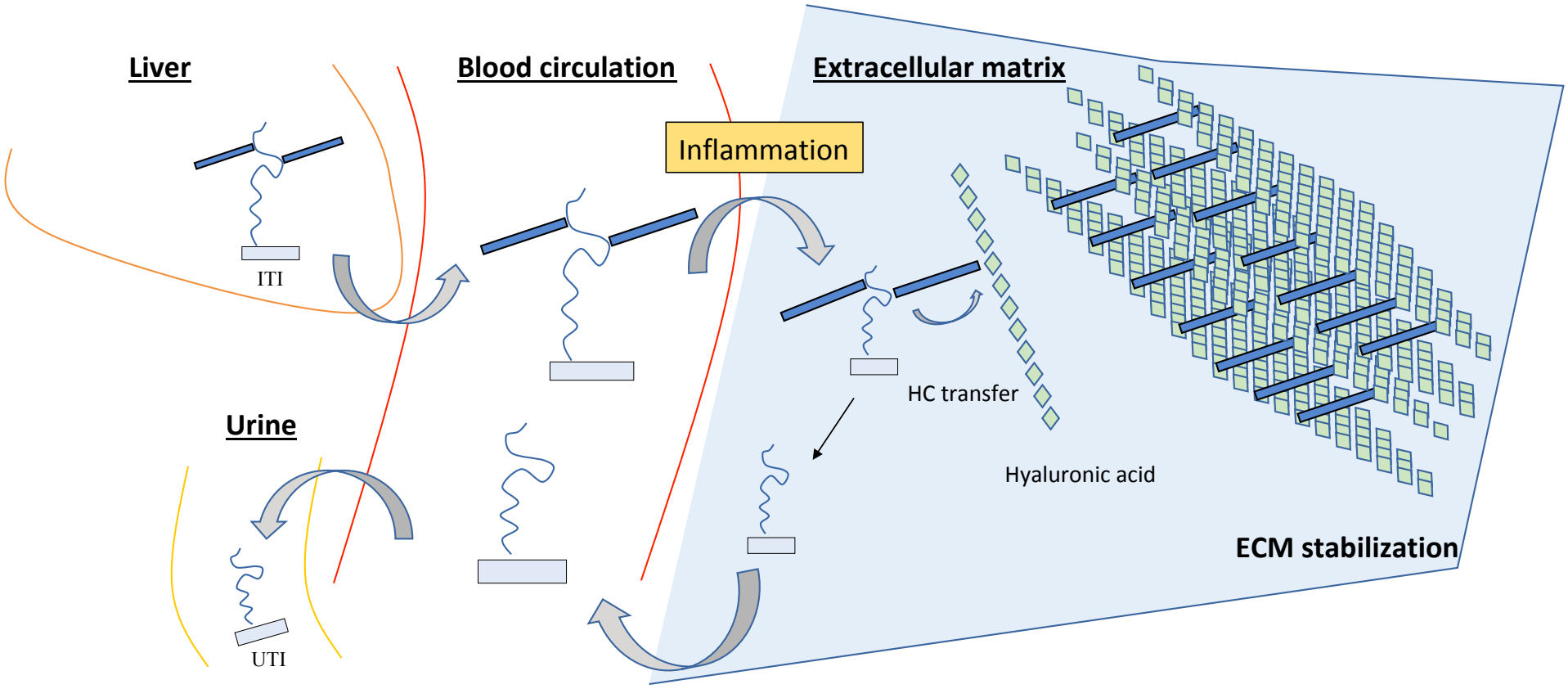
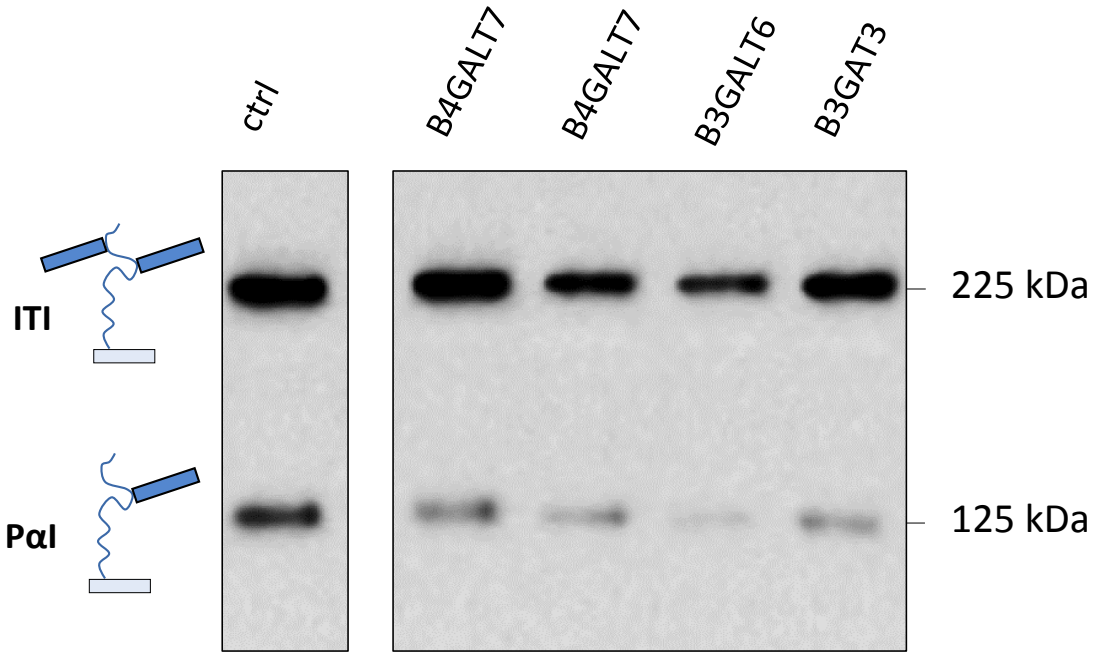
Major physiological role of Bikunin isoforms



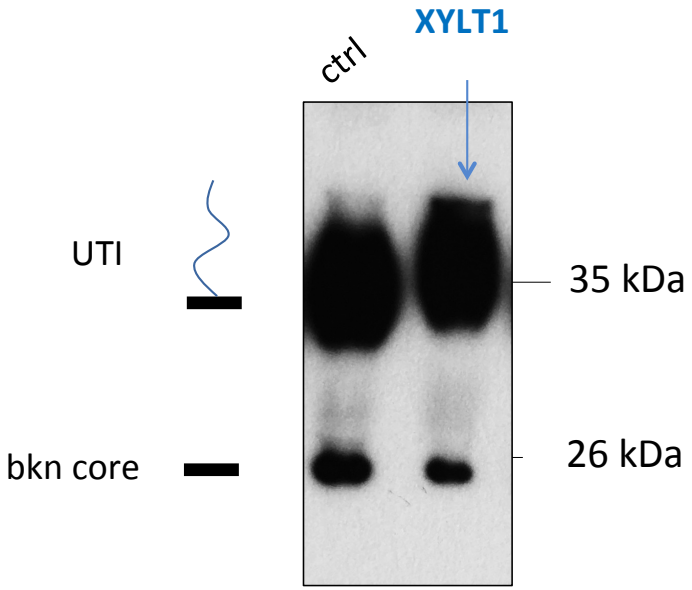
Heavy forms in linkeropathy patients are normal



Correct elongation despite the enzymatic defect

Light forms in XYLT1 patient are normal

XYLT1 is not expressed by the liver !



Limited to mutations altering proteins from liver origin

What could be a convenient biomarker of linkeropathies ?

As far as possible…

Structure carrying a GAG chain containing the tetrasaccharide linker

- Proteoglycan

Circulating proteoglycan

- Availability in blood/serum/plasma/urine samples
- Simple and sensitive detection (antibodies)

Specificity/sensitivity

- No missing diagnostic
- No wrong positives
- Ability to discriminate between each mutation

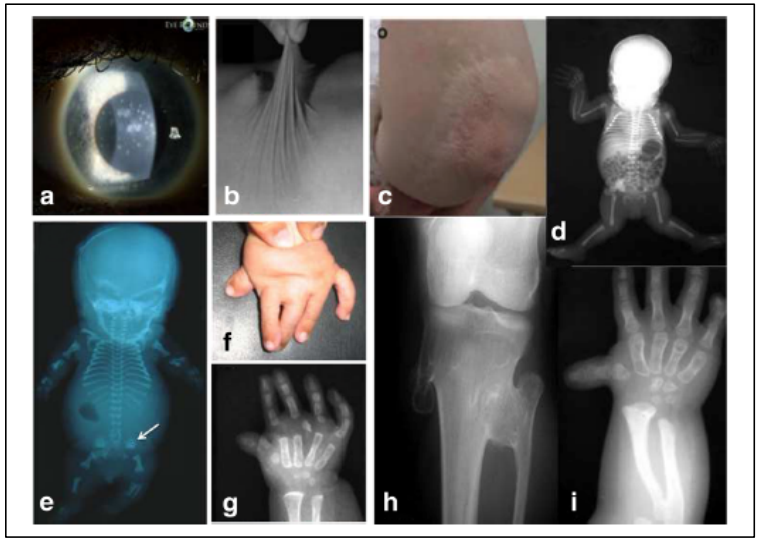
Bikunin proteoglycan

Screening & diagnosis of linkeropathies

Clinical approach

- Recurrent symptoms
  - Short stature (dwarfism)
  - Joint laxity/dislocations
  - Dysmorphic features
  - Cutaneous abnormalities (skin hyperlaxity)

- Unspecific symptoms
  - Ocular diseases (blue sclerae), deafness
  - Cardiac diseases



Tough biochemical diagnosis

Lack of blood biomarkers

- Enzyme activity measurements (patients fibroblasts, substrates, mRNA levels)
- GAG quantification (patient fibroblasts, ^{35}S incorporation assays, IHC)
- Mass spectrometry following HPLC
- Gene panel sequencing, whole exome sequencing

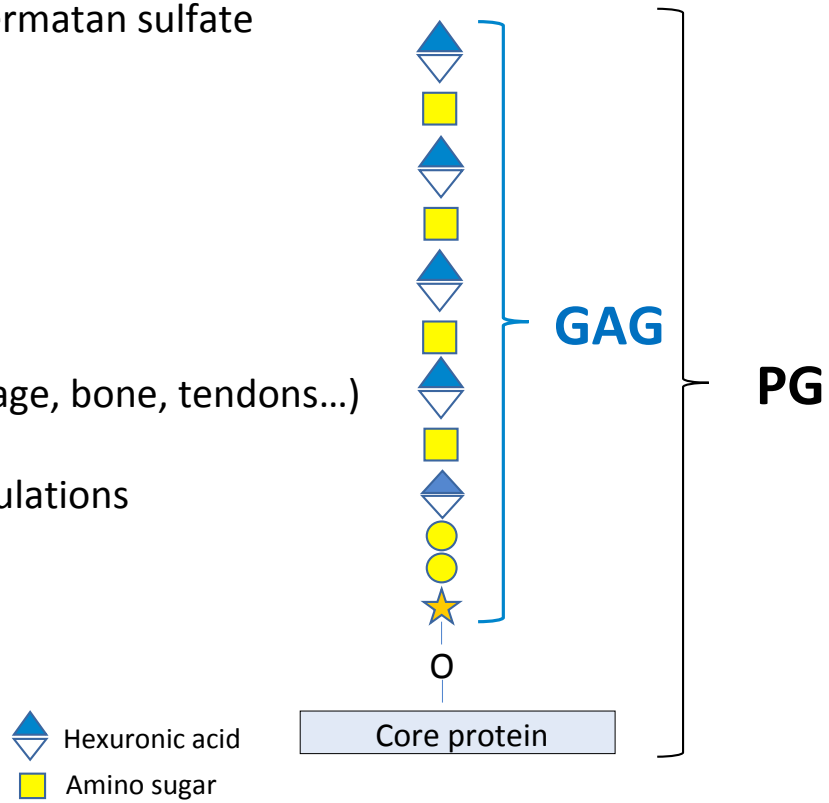
Glycosaminoglycans (GAG) / Proteoglycans (PG)

- GAG = **polysaccharides** = macromolecules composed of repeated carbohydrate units
  ex: heparin/heparan sulfate, chondroitin sulfate, dermatan sulfate

- PG = **protein** linked to a GAG
  ex : perlecan (HS), decorin (DS), versican (CS)…

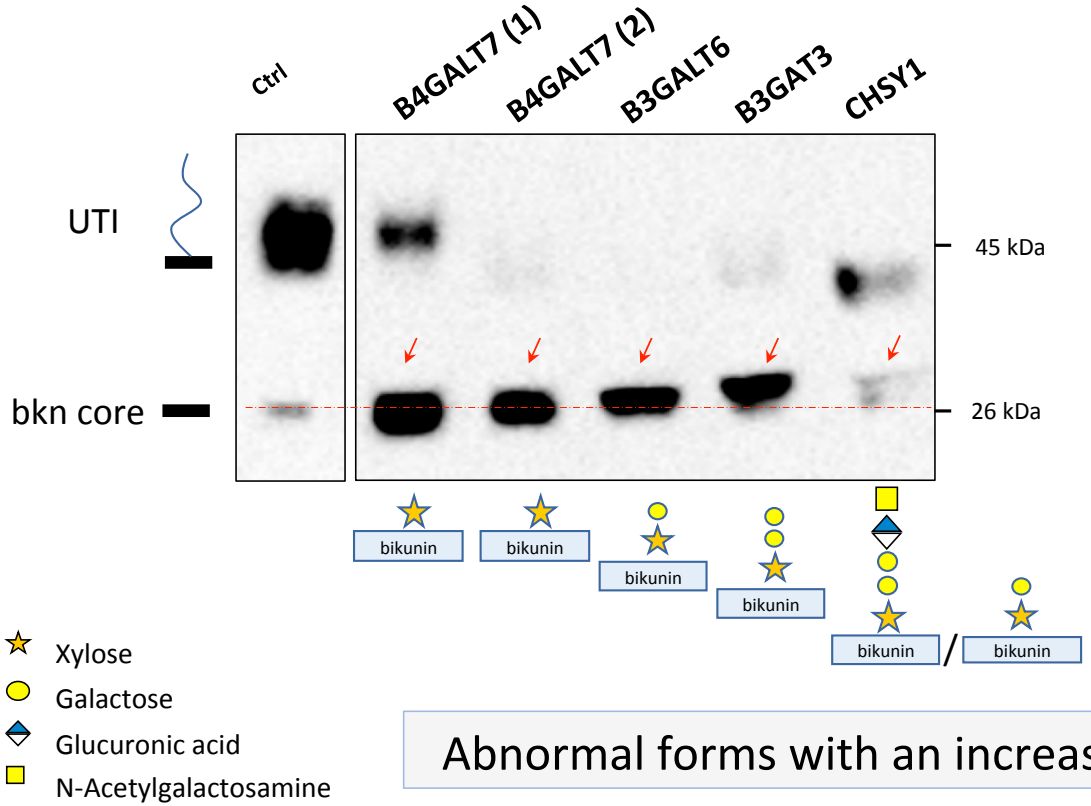
- Structural constituents
  - ➢ Major component of the extracellular matrices (cartilage, bone, tendons…)
  - ➢ Crucial role in the architecture and properties of articulations

- Signaling molecules
  - ➢ Regulation of growth development

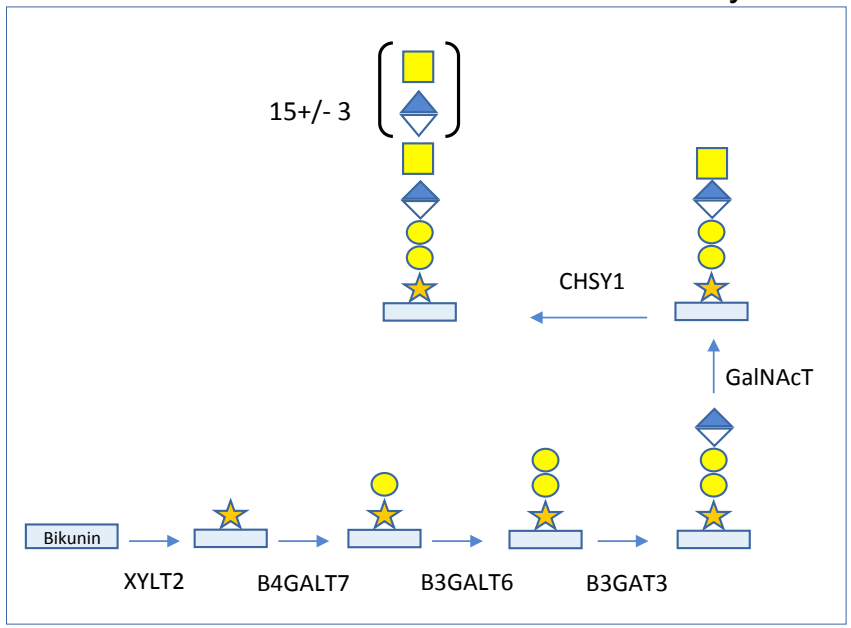


Serum bikunin **light forms** analysis in linkeropathies patients (1)

Western-blot using anti-bikunin antibodies



"Linker" and chondroitin sulfate GAG biosynthesis



Abnormal forms with an increasing molecular weight