Two affected siblings with a shared ATP6AP2 de novo intronic X-linked mutation. Novel splicing alteration and first described female ATP6AP2-CDG case

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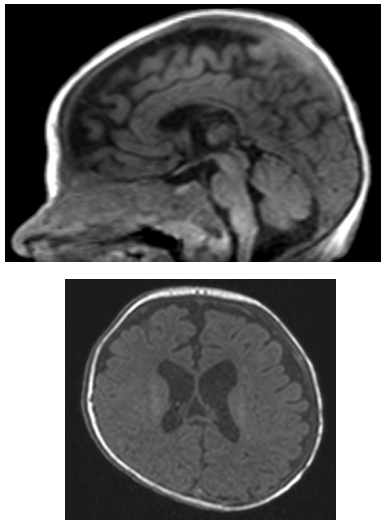
EUROGLYCAN Network meeting, Prague, 19-21 June 2023

Clinics

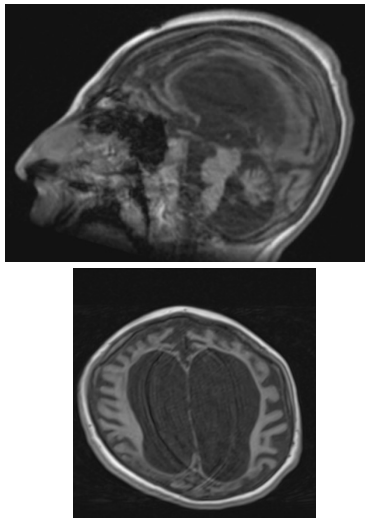
- Two affected siblings of Caucasian origin; unrelated parents
- **Girl** (12 y.o): mild intellectual disability (ID), autism, and progressive microcephaly
- **Boy** (8 y.o): major ID with absence of psychomotor development. Severe neonatal epileptic encephalopathy, axial hypotonia, diaphragmatic paralysis, axonal neuropathy and progressive microcephaly. Brain MRI: progressive cortico-subcortical atrophy; progressive cerebellum atrophy
- No liver involvement; normal coagulation factors; normal cooper and ceruloplasmin. Unexplained hypokalemia in the boy

Brain MRI (boy)

3 months



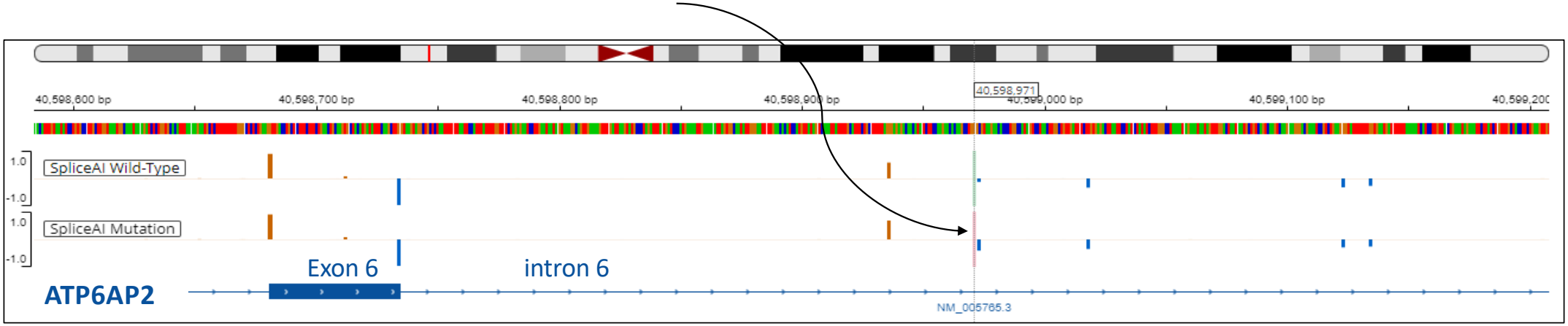
6 years



Genetics

Hôpital Pitié-Salpêtrière AP-HP

- Quatuor genome sequencing ➔ intronic variant: **ATP6AP2:c.588+237G>A** on chrX; shared by the 2 patients



- Not found in general population (gnomAD, deCAF ~300.000 ind.); heterozygous in the sister, hemizygous in the brother;
- *De novo* variant thought to result from **germinal mosaicism in the mother**

Glycosylation

Capillary electrophoresis of serum transferrin

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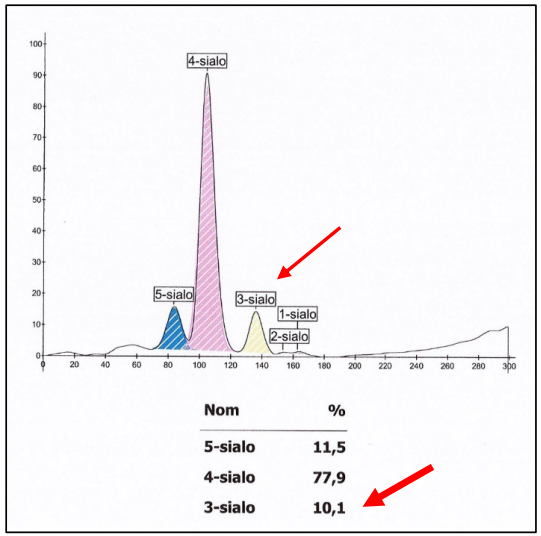
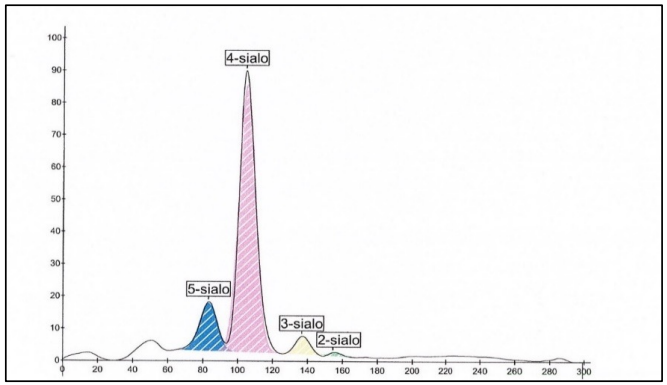
Girl

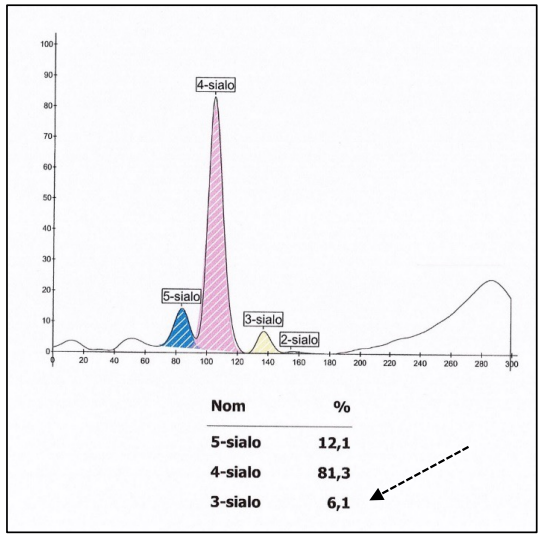


control



5-sialo : 11.3%
4-sialo : 82.3%
3-sialo : < 6%

Boy

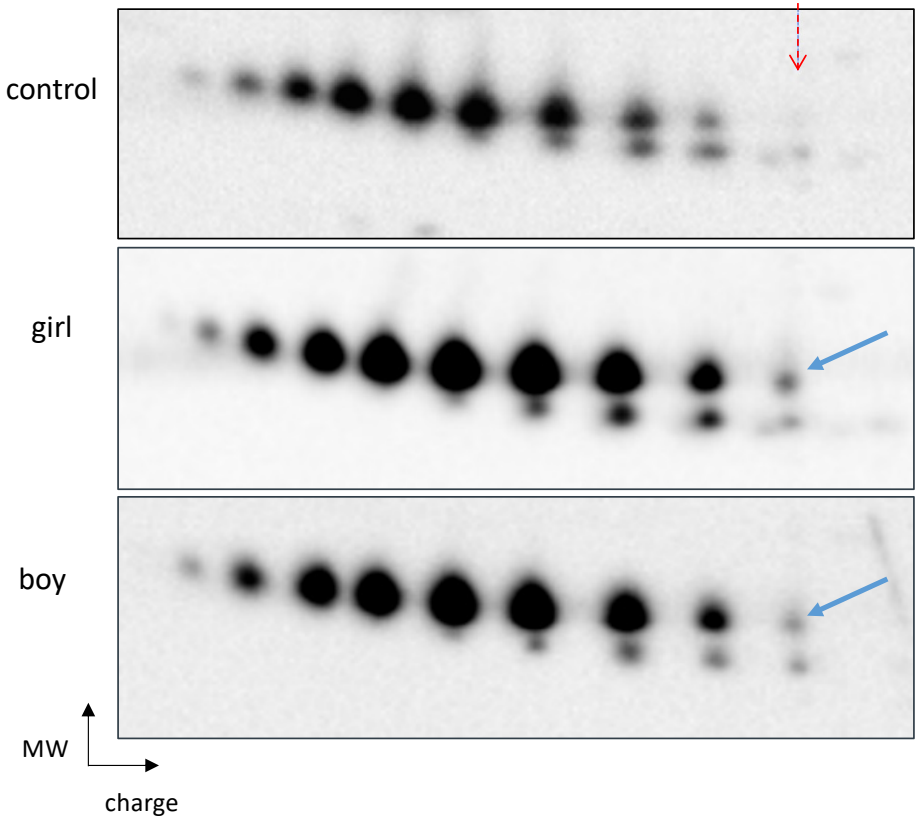


➔ Discrete **CDG-II** profiles

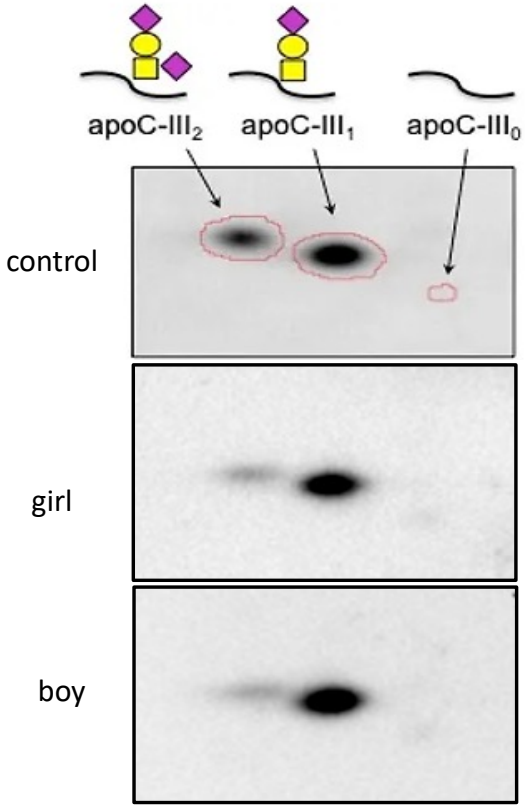
Glycosylation

Haptoglobin and apoC-III

2D electrophoresis of de haptoglobin
(N-glycosylation)



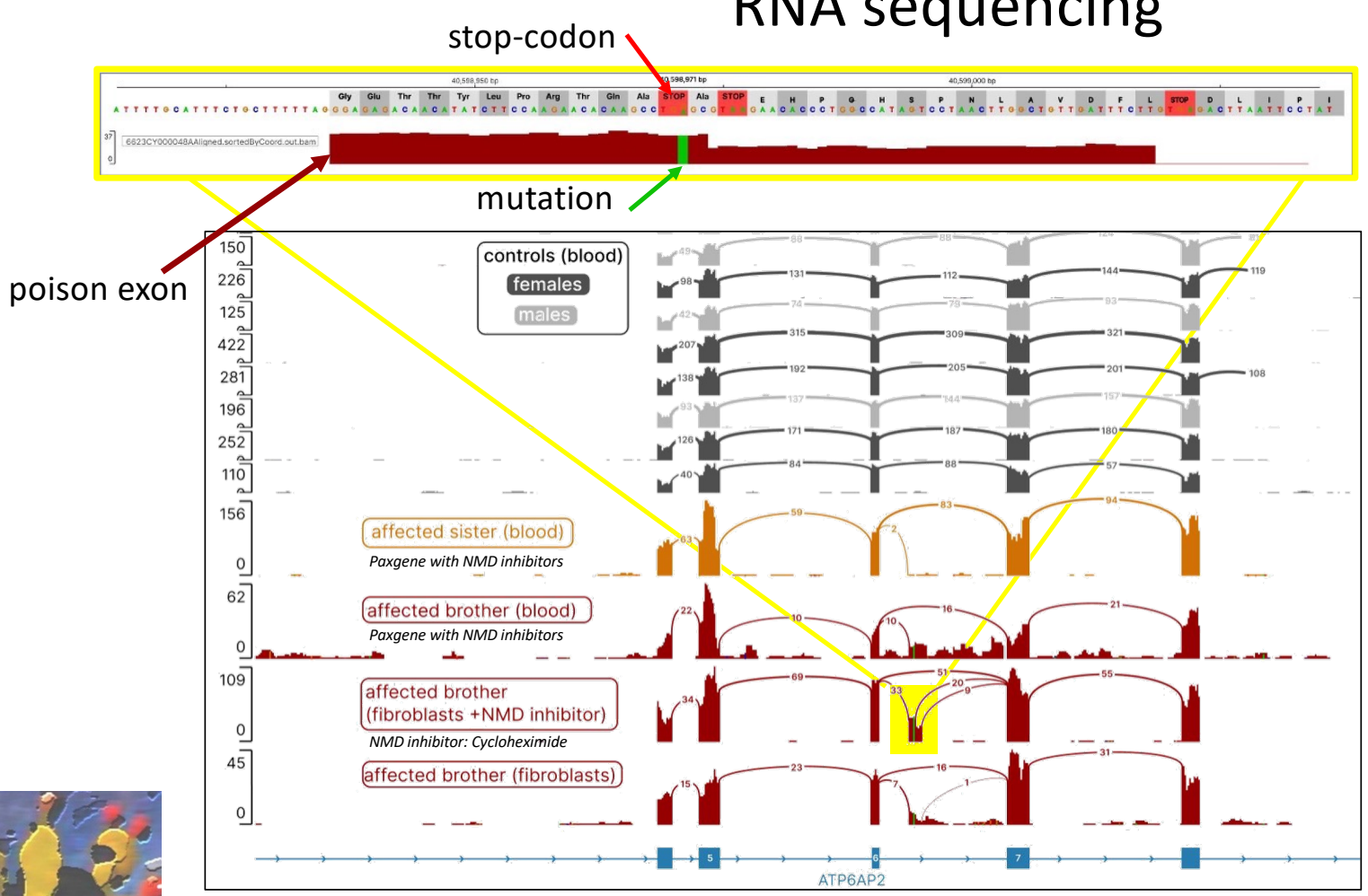
2D electrophoresis of apoC-III
(O-glycosylation)



Typical
« apoC-III_1 » patterns

➔ Combined N- and O-glycosylation defects

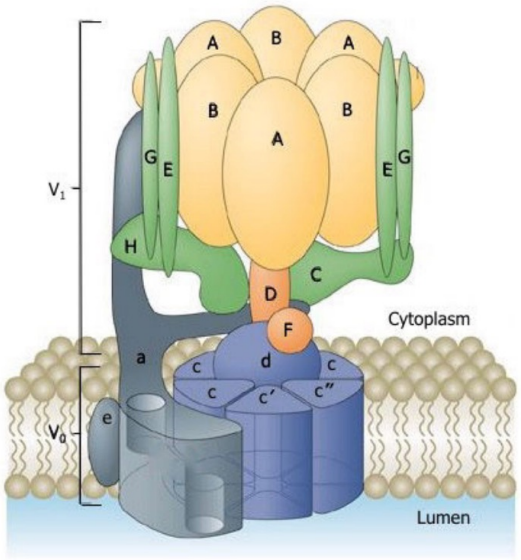
RNA sequencing

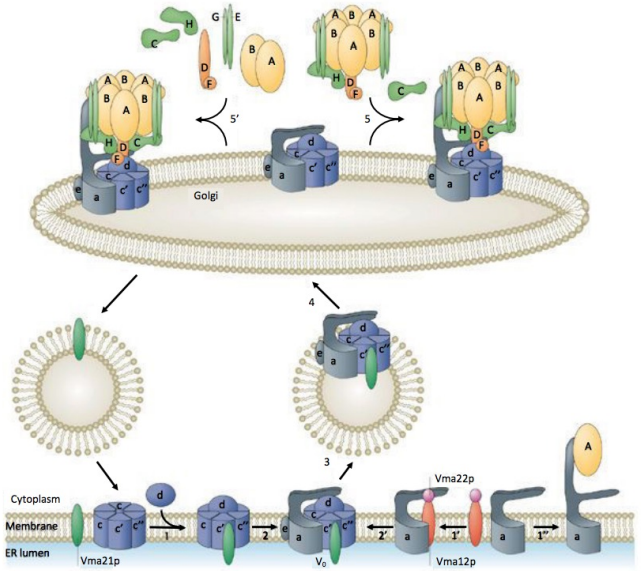


- Partial but **severe splicing alterations** in the boy (blood cells and fibroblasts); **pseudoexonisation** of poison intronic sequence containing a **premature stop-codon.**

- No anomalies in the girl (blood cells)
- **Skewed X inactivation** (18 % / 82 %)

V-ATPase and CDG





- ATP6V0A2-CDG : neurological and cutaneous phenotype
- CCDC115-CDG : liver phenotype (Wilson-like) +/- neurological
- TMEM199-CDG: liver phenotype ++ (Wilson-like) +/- neurological

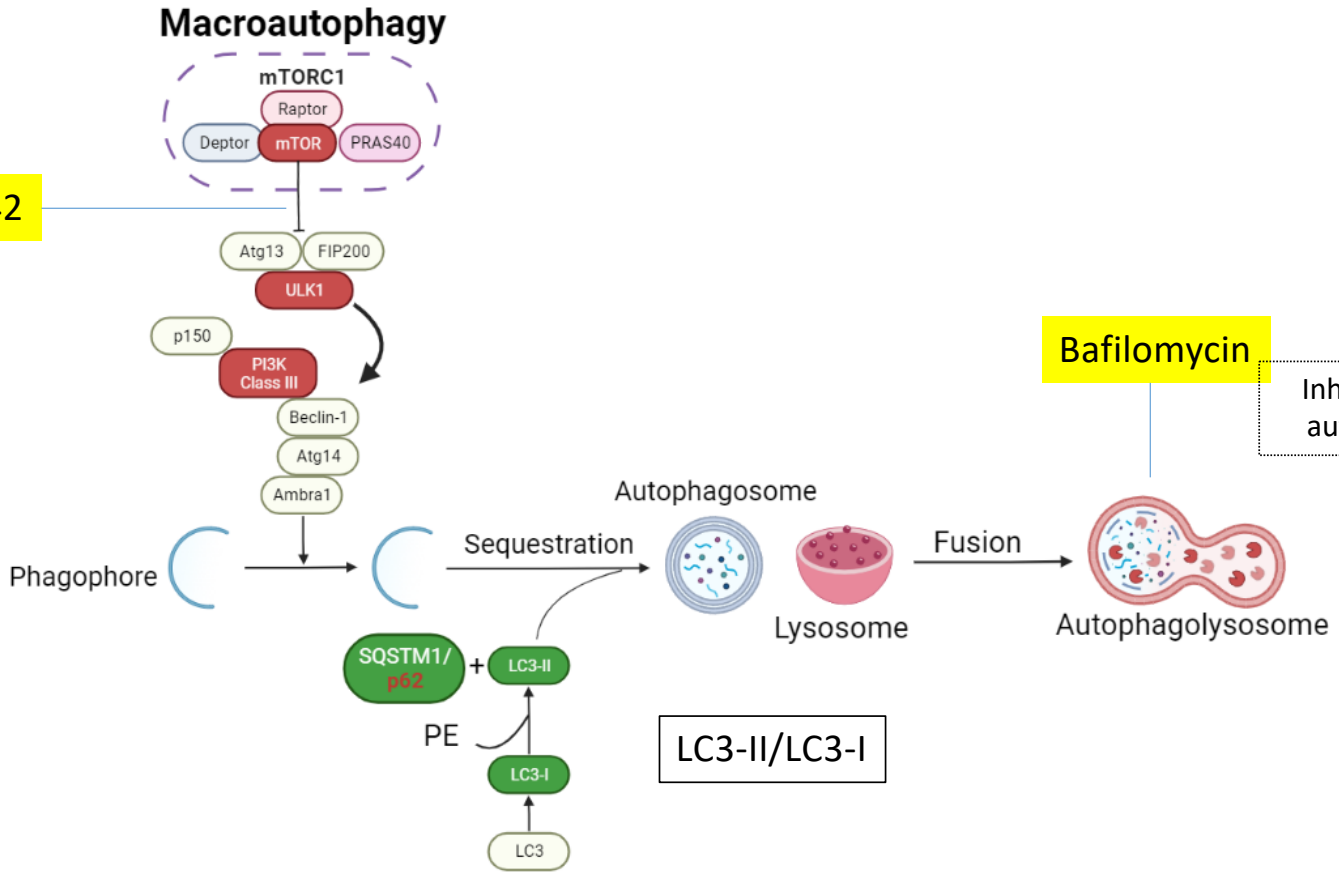
- ATP6AP1/2-CDG : liver (Wilson-like) AND/OR neurological phenotype; autophagy and acidification defects

*- Rujano MA, et al. Mutations in the X-linked ATP6AP2 cause a glycosylation disorder **with autophagic defects.** J Exp Med. 2017*
*- Hirose T, et al. ATP6AP2 variant impairs CNS development and neuronal survival to cause **fulminant neurodegeneration**. J Clin Invest. 2019.*

Autophagy

Stimulation of the formation of autophagosomes

PP242

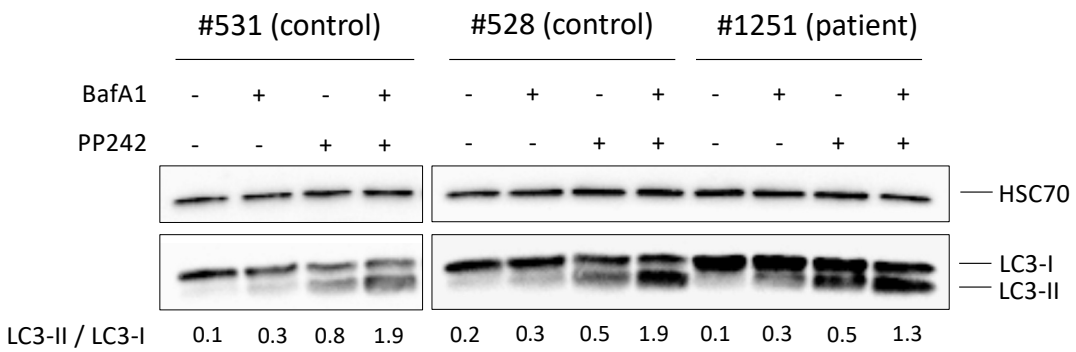


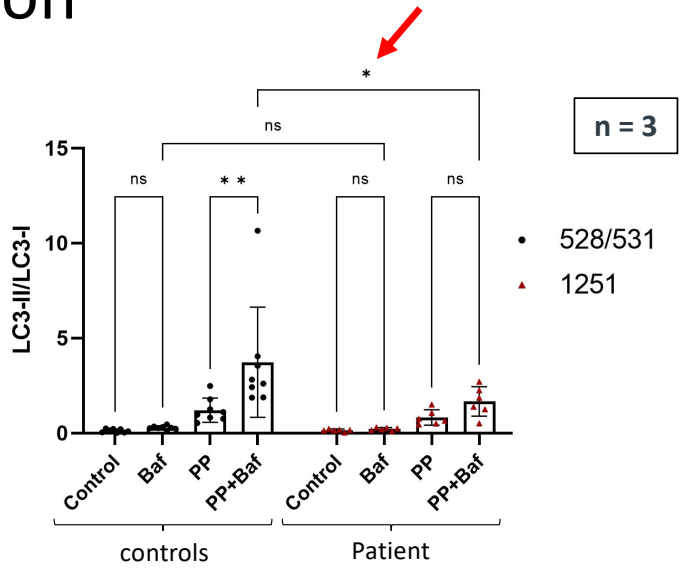
Bafilomycin

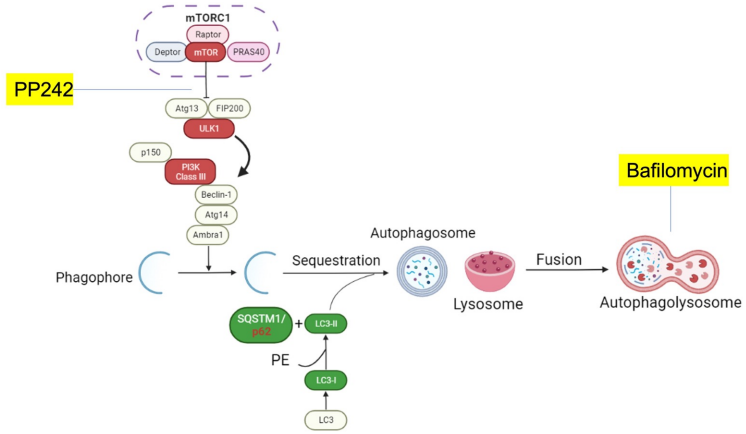
Inhibition of the autophagic flux

LC3-II/LC3-I

Autophagy and acidification







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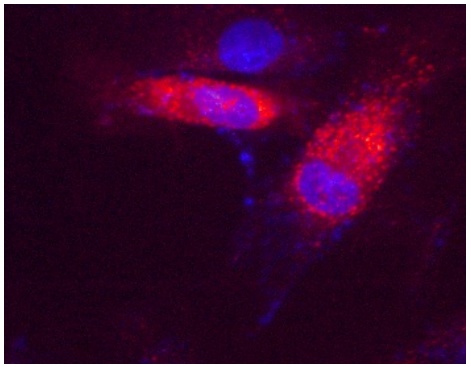
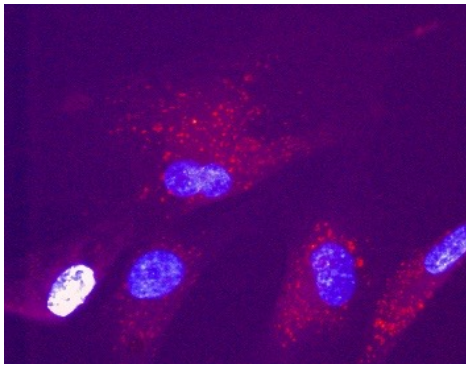


patient



Lysotracker

Conclusions

- Two affected individuals with neurological clinical phenotypes
- New *de novo ATP6AP2* intronic variant
- CDG-II with Golgi homeostasis deficiency
- Mutation leading to partial pseudoexonisation and stop-codon
- Associated to autophagy and acidification defects
- First female ATP6AP2-CDG case?

- Western-blot of ATP6AP2 protein

THANKS!

Jean-Madeleine de Sainte Agathe

Magalie Barth

Sophie Cholet
François Fenaille

Elodie Lebredonchel
Arnaud Bruneel
Katell Peoc'h

Paola Bellenda
Alexis Couasnard
Elise Jacquin
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Ameetha Ratier

www.CDG-Bichat.com

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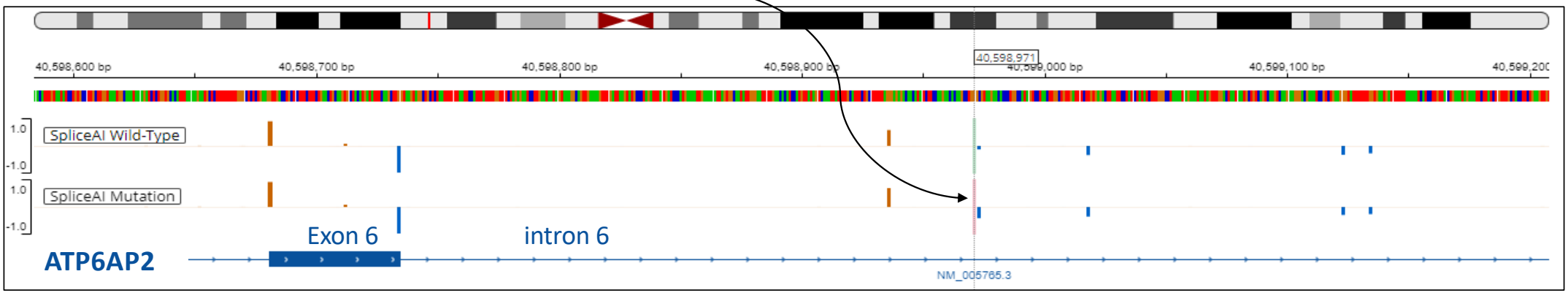

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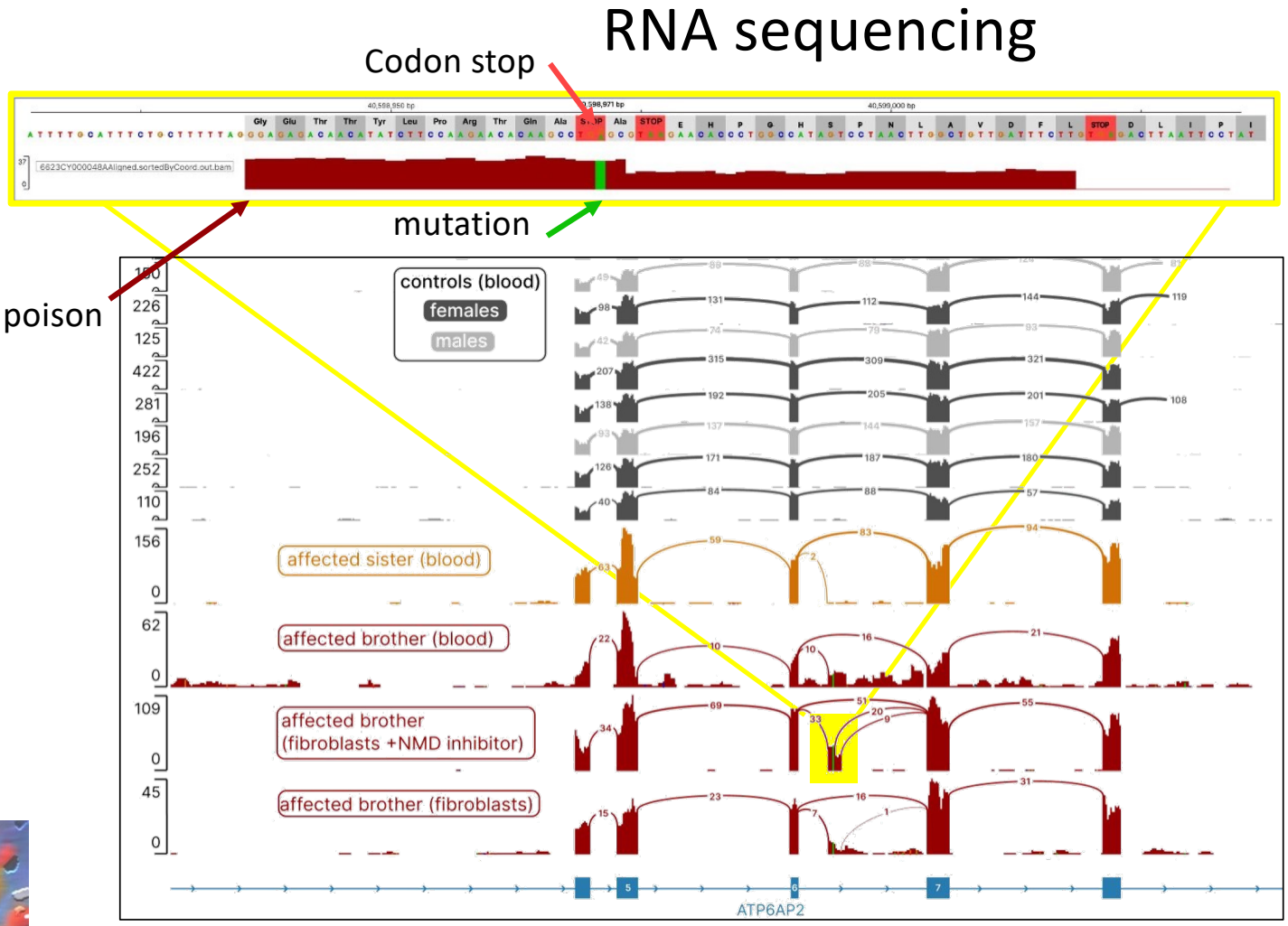

Il s'agit du nombre de read (NGS) qui chevauche la jonction exon/exon, et qui sont écartelés lorsqu'ils sont alignés sur un génome de référence.

Effectivement, ça semble plus propre. Ce qui ne me semble pas super logique, je te l'accorde. En revanche, ce qui est tout à fait intéressant (et plus logique), c'est de voir que la proportion de l'exon poison augmente, lorsque le NMD est inhibé.

La proportion de transcrit normal peut être estimée via la proportion de jonctions normales (chez la sœur, en orange, 83) versus le nombre de jonctions aberrantes (2 chez la sœur)
Donc du point de vue proportionnel, le frère a moins voire beaucoup moins de transcrit normal.

Théoriquement, tous les contrôles sont des ARN sanguins en situation d'inhibiteur NMD (c'est ce que prétend le fournisseur des tubes PaxGene, en tout cas), mais ce n'est pas comparable avec la culture de fibroblaste, et la véritable inhibition du NMD qu'on a faite grâce à l'ajout de cycloheximide.