Modulators of neuroinflammation have a beneficial effect in Lafora disease

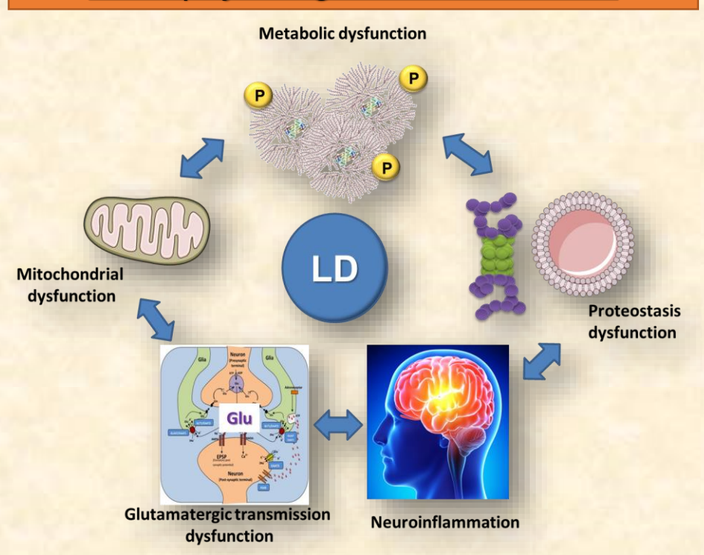
B. Mollá[1,2], M. Heredia[1,2], M.A García-Gimeno[3] and P. Sanz[1,2]

1.- Laboratory of Nutrient Signaling, Institute of Biomedicine of Valencia (CSIC), Valencia, Spain;
2.- U742 CIBER de Enfermedades Raras (CiberER), Spain; 3.- Dept. Biotechnology, ETSIAM, Polytechnic Univ. Valencia, Spain

ABSTRACT

- ✓ **Lafora disease (LD)** is a rare human disorder (OMIM#274780) showing a severe **epileptic** and **neurodegenerative** status with tonic clonic **seizures, memory deficits** and a **rapid neurological decline.**
- ✓ The onset is in **adolescence** and leads to **death** of the patient **within 10 years,** because an efficient treatment is still missing.
- ✓ The hallmark is the accumulation of insoluble polyglucosans, called **Lafora bodies (LBs)**, typically in brain.
- ✓ Mutations in either **laforin (*EPM2A*)** or **malin (*EPM2B*)** genes cause the disease, and are clinically indistinguishable.
- ✓ Loss of function of laforin or malin have been related to metabolic dysfunction, proteostasis and autophagy impairment(1,2), neuroinflammation(3), deficient glutamatergic transmission(4) and an oxidative stress status(5) in mouse models of LD.
- ✓ Using a malin-deficient mice **(*Epm2b*$^{-/-}$)** we are testing **different pharmacological approaches** in order to assess their efficacy ameliorating the pathological phenotype present in this mouse model of LD (6).

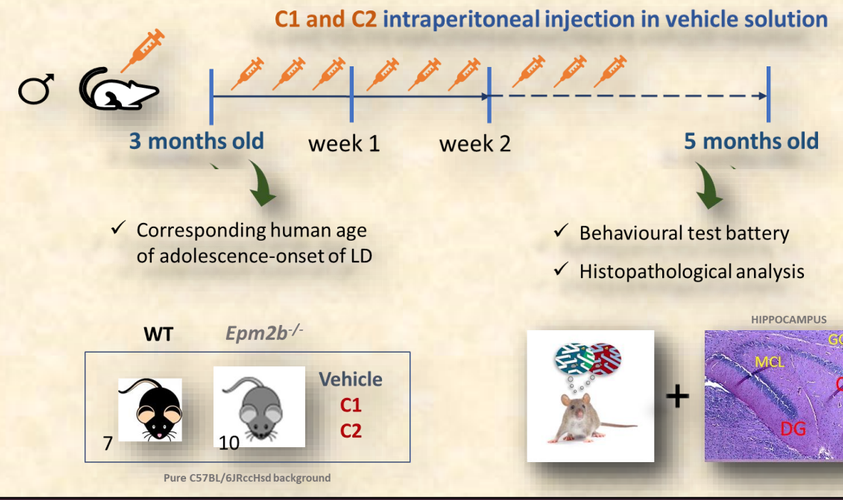
Pathophysiological mechanism (7)



METHODS

- ✓ ***Epm2b*$^{-/-}$** mice received treatment with either **C1** or **C2** compounds whose main therapeutic mechanism is to **modulate neuroinflammation**.
- ✓ We injected intraperitoneally vehicle, C1 and C2 compounds in **Epm2b$^{-/-}$** (N=10 per group) and corresponding **WT** mice (C57BL/6JRccHsd; N=7 per group) **from 3 to 5 months of age.**
- ✓ Next, we performed a **battery of behavioral tests** and **histopathological analyses** to evaluate whether modulating neuroinflammation has a therapeutic effectiveness in LD.
- ✓ On the whole, this work shows a **preclinical study of modulators of neuroinflammation in *Epm2b*$^{-/-}$ mice** as a novel pharmacological strategy in LD.

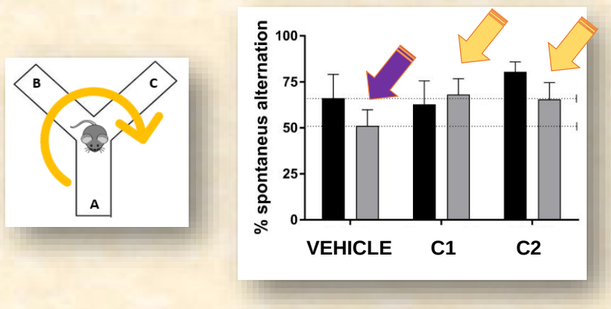
Pharmacological treatment timeline



RESULTS

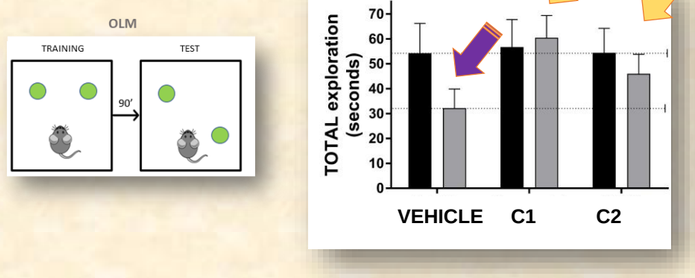
1. BEHAVIOURAL PHENOTYPING: COGNITIVE AND NEURODEGENERATIVE STATUS

A) WORKING MEMORY TEST (Y-MAZE)



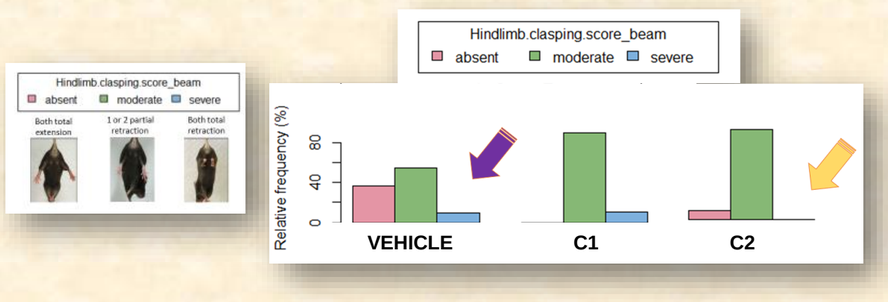
A) Relative proper spontaneous alternation between three arms (ABC) was tested using a Y-maze and related to working memory skill.
Epm2b$^{-/-}$ mice showed a working memory defect that was reversed by C1 and C2 treatments

B) OBJECT LOCATION MEMORY TEST (OLM)



B) Total exploratory time of objects was measured using an object location memory test and related to attention capacity.
Epm2b$^{-/-}$ mice showed an attention defect that was reversed by C1 and C2 treatments

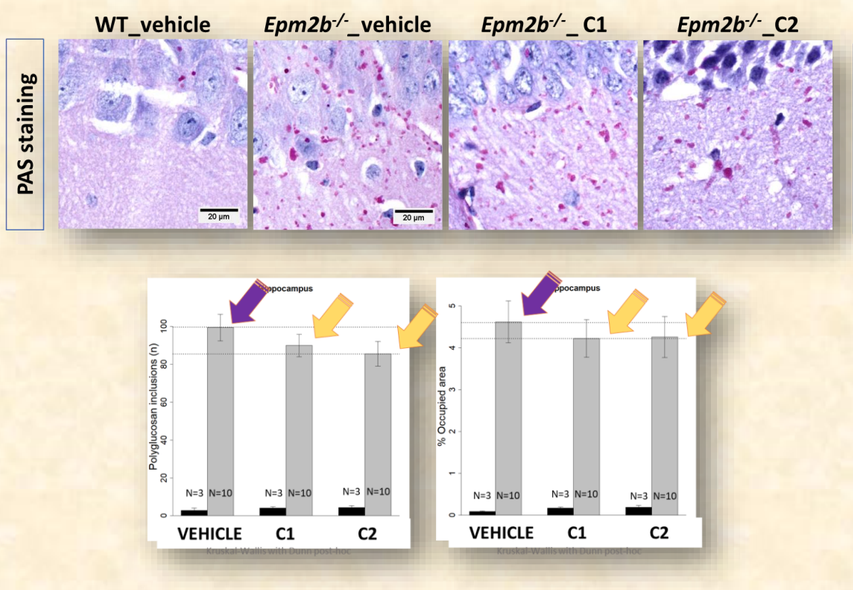
C) NEURODEGENERATIVE STATUS (Hindlimb clasping)



C) The degree of hindlimb clasping was scored from absent to severe and related to neurodegenerative status. Some *Epm2b*$^{-/-}$ mice showed a severe score that was reversed by C2 treatments

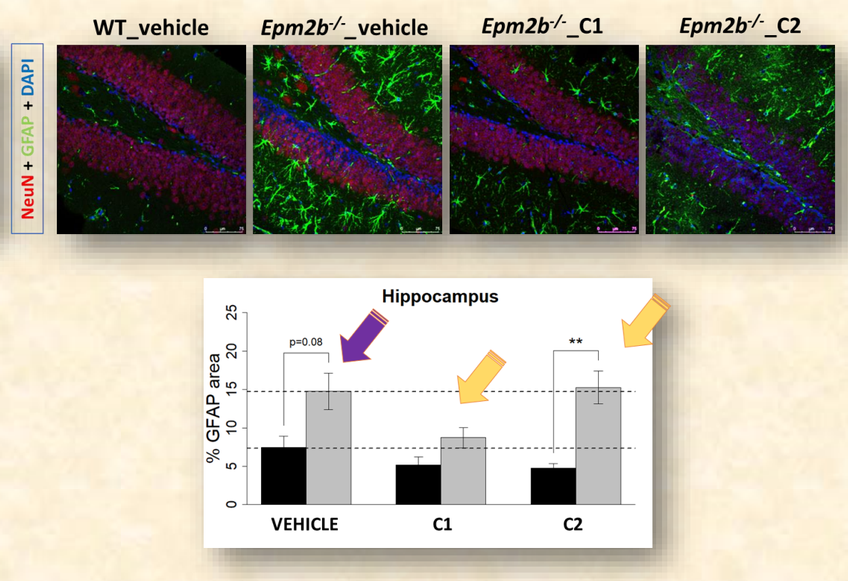
2. HISTOPATHOLOGICAL ANALYSIS IN HIPPOCAMPUS

A) POLYGLUCOSAN INCLUSIONS



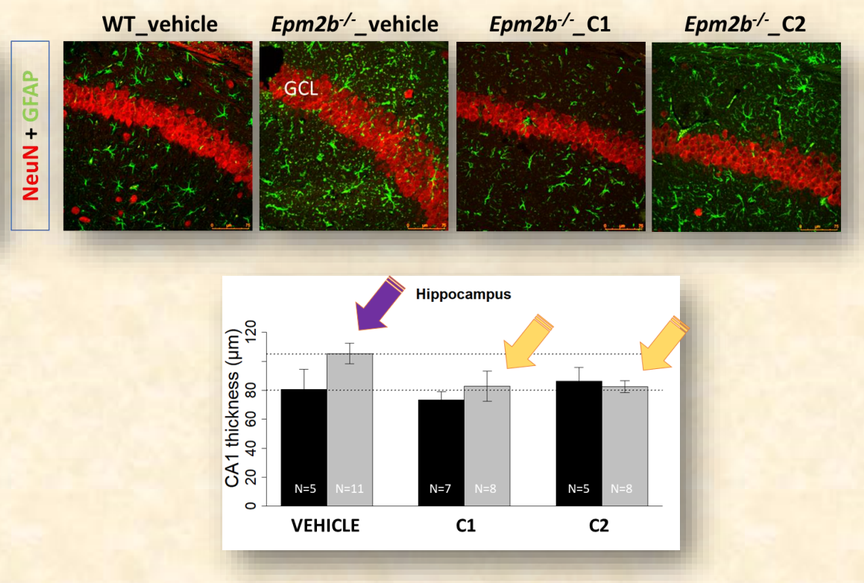
A) Number and % occupied area by polyglucosan inclusions (in pink) were detected by PAS staining *Epm2b*$^{-/-}$ mice significantly showed a huge amount of polyglucosan inclusions which was slightly reversed by C1 and C2 treatments

B) ASTROGLIOSIS



B) % GFAP area (in green) was detected by immunofluorescence and related to a pathological astrogliosis condition.
Epm2b$^{-/-}$ mice significantly showed astrogliosis which was reversed only by C1 treatment

C) GRANULAR CELL LAYER (GCL)THICKNESS



C) Granular cell layer thickness (GCL) (in red) was detected by immunofluorescence and related to a pathological dispersion of neurons.
Epm2b$^{-/-}$ mice showed a dispersion in GCL which was reversed by C1 and C2 treatments

CONCLUSIONS

We have found that **modulating neuroinflammation** using **C1 and C2** compounds has a **therapeutic effectiveness in LD mouse model** since:

- ✓ **C1 and C2** compounds tend to **improve a working memory and an attention defect** showed by *Epm2b*$^{-/-}$ mice
- ✓ **C2** compound is able to **ameliorate the severe scores of neurodegenerative status** found in *Epm2b*$^{-/-}$ mice
- ✓ Both **C1 and C2** compounds not only slightly **reduce the amount of polyglucosan inclusions** but also considerably **reduce the dispersion of GCL in hippocampus**
- ✓ **C1** is able to **decrease remarkably astrogliosis condition**

REFERENCES

(1)Aguado et al., 2010, *Hum. Mol. Genet*; (2) Knetch et al., 2012, *Autophagy*; (3) López-González et al., 2015, *Mol Neurobiol*.; (4) Muñoz-Ballester, C et al, 2016, *BBA Molecular Basis of Disease*; (5) Romá-Mateo et al., 2015, *Free Radical Biology and Medicine*; (6) Berthier et al, 2016, *Mol Neurobiol*; (7) García-Gimeno MA et al. , 2018, *Cells.*