Characterization of 4L/PS-NA mice for different biomarkers to model Gaucher disease

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BACKGROUND

Gaucher disease is the most common lysosomal storage disease. The neuronal disease variant is characterized by protein accumulations in the brain and associated neurological manifestations. It is autosomal recessively inherited and modeled by 4L/PS-NA mice that express low levels of prosaposin and saposins, as well as a functionally impaired β-glucosidase (GCase) with a homozygous point mutation at V394L.
To use this model for compound tests against Gaucher disease a detailed characterization of these mice is needed. Thus, we analyzed the 4L/PS-NA mice for GCase activity, glucosylsphingosine (GlcSph) and glucosylceramide (GlcCer) levels as well as Neurofilament light chain (NF-L) levels and neuroinflammation over age.

MATERIALS and METHODS

The GCase activity was analyzed using a commercially available GCase activity assay. GlcSph and GlcCer were extracted from brain homogenates by liquid-liquid extraction and the levels of GlcSph and GlcCer in the extracts were measured by ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry. To explore Neurofilament light chain levels, the NF-Light® ELISA by UmanDiagnostics was used. For measurement of neuroinflammatory processes, in particular activated microglia and astrocytosis, immunofluorescent labeling on brain sections was performed.

RESULTS

Analyses of enzyme activity show reduced GCase levels in 4L/PS-NA and also 4L/PS+/+NA mice compared to C57Bl/6 mice. The additional reduction of prosaposin and saposins leads to progressively increasing substrate concentrations in 4L/PS-NA mouse brains compared to 4L/PS+/+NA animals. Furthermore, 4L/PS-NA mice show strongly increased NF-L and neuroinflammation levels.

RESULTS

Neurofilament Light Chain

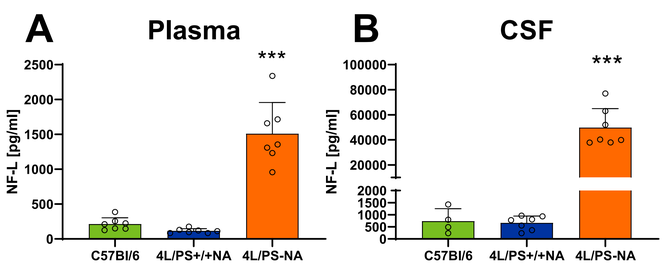

Figure 1. Quantification of neurofilament light chain in plasma and CSF of 4L/PS-NA mice of mixed sex. **A**: NF-L levels in pg/ml in the plasma of 18 week old 4L/PS-NA mice compared to 4L/PS+/+NA littermates and C57Bl/6 mice **B**: NF-L levels in pg/ml in the CSF of 18 week old 4L/PS-NA mice compared to C57Bl/6 and 4L/PS+/+NA littermates. One-way ANOVA with Tukey's multiple comparison test. n = 7. Mean + SEM. ***p<0.001 compared to C57/Bl6 and 4L/PS+/+NA animals.

GCase Activity and Substrate Levels

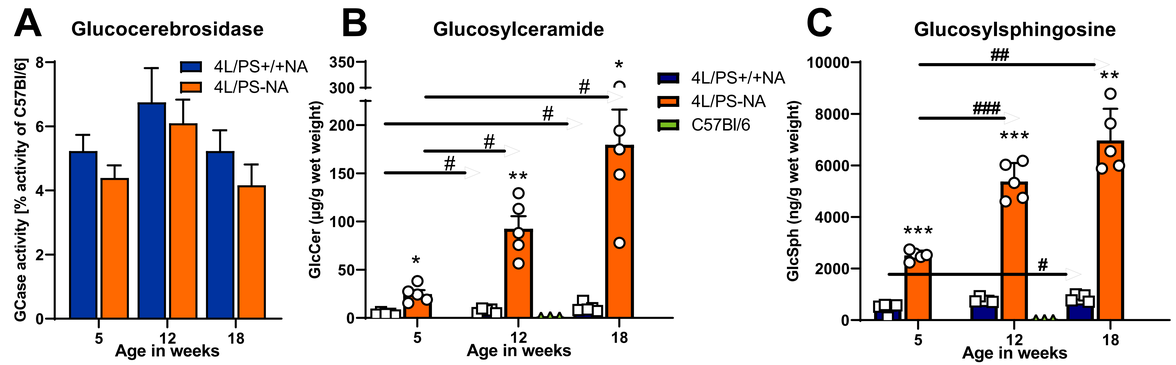

Figure 2. Quantification of cerebral GCase activity, Glucosylceramide and Glucosylsphingosine in 5 to 18 week old 4L/PS-NA mice. **A**: CBE inhibitable GCase ativity measured with 4-MUG assay. **B**: Glucosylceramide in µg/g wet weight. **C**: Glucosylsphingosine in ng/g wet weight. **B** and **C**: 12 week old C57Bl/6 mice were added as additional control group but this group was excluded from statistical analysis. Mean + SEM; n = 5; *differences between genotypes; # differences between age groups; *p<0.05; **p<0.01; ***p<0.001.

Neuroinflammation

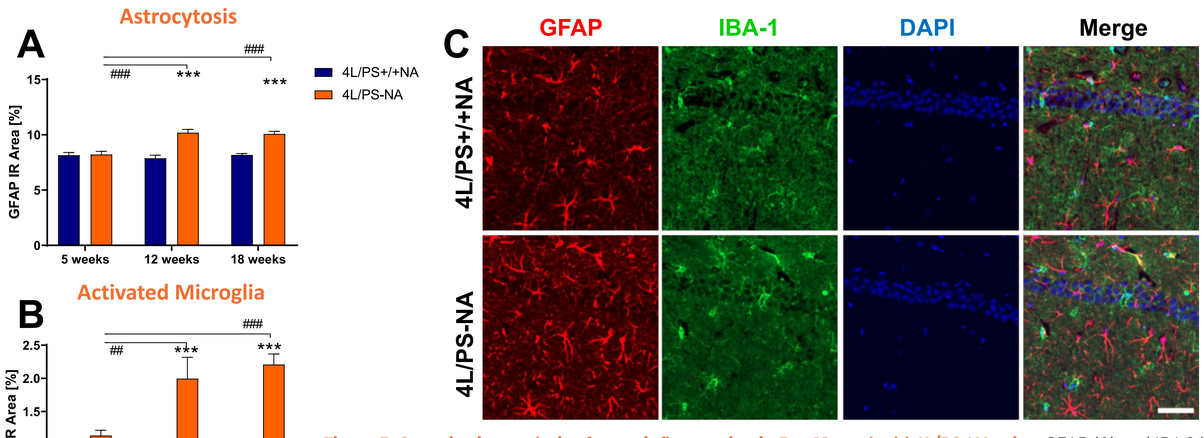

Figure 3. Quantitative analysis of neuroinflammation in 5 to 18 week old 4L/PS-NA mice. GFAP (**A**) and IBA-1 (**B**) immunoreactive (IR) area in percent in the hippocampus of 5, 12 and 18 week old 4L/PS-NA mice compared to 4L/PS+/+NA littermates. Two-way ANOVA with Bonferroni's *post hoc* test. Mean + SEM; n=5 per group; *differences between genotypes; # differences between age groups; **p<0.01; ***p<0.001. **C**: Representative images of GFAP (red), IBA1 (green) and DAPI (blue) labeling of the hippocampal CA1 region in 18 week old 4L/PS-NA and 4L/PS+/+NA mice. Scale bar: 50 µm.

SUMMARY and CONCLUSION

4L/PS-NA mice mimic the most prominent features of Gaucher disease suggesting that they are a good model to study the chronic neuronopathic type 3 Gaucher disease in humans.

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For more information about the model please visit:
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