Sigma-1 receptor agonists as potential therapies for CLN6 Batten disease

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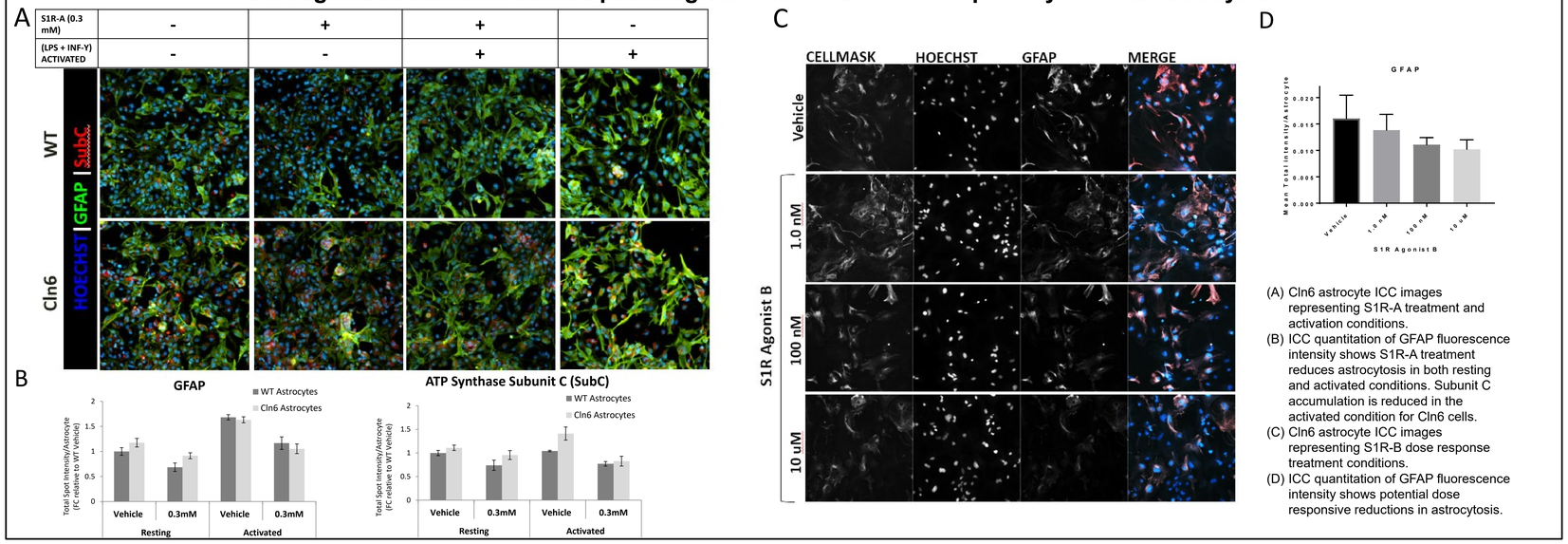
Abstract

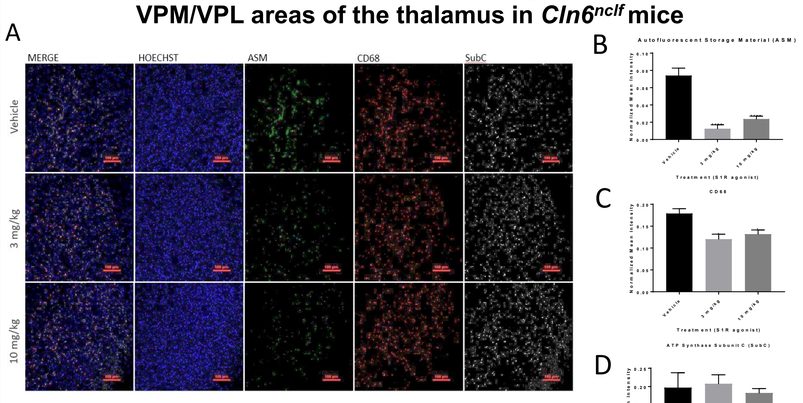
CLN6 Batten disease is a rare, late infantile onset neuronal ceroid lipofuscinosis (NCL) subtype characterized by the abnormal accumulation of lysosomal materials, progressive neurodegeneration, loss of motor function, seizures, impaired speech, vision loss, and death. Work in other neurodegenerative models, such as Rett syndrome and Alzheimer's disease, have demonstrated a role for the molecular chaperone sigma-1 receptor (S1R) in slowing disease pathogenesis. Here, we have used *in vitro* primary culture and a mutant mouse model of CLN6 disease (*Cln6*^{nclf}) to evaluate the efficacy of an S1R agonist in disease pathogenesis. *In vitro* analyses demonstrated S1R agonists attenuate both gliosis phenotypes and lysosomal storage material in CLN6 astrocytes. To evaluate the *in vivo* impact of S1R activity, *Cln6*^{nclf} mice were analyzed for CLN6 hallmark pathology and neurological function following 90 day administration of an S1R agonist (s.q.10 mg/kg or 3 mg/kg daily). Significant reductions in auto-fluorescent storage material, the microglial marker CD68, and the CLN6 marker ATP synthase subunit C were observed across various brain regions, including within the thalamus, cerebral cortex, and cerebellum. However, neuropathological changes varied across tissues, suggesting possible region-specific impacts of S1R activity in CLN6. Western blot analysis of micro-dissected *Cln6* and control CNS tissue revealed a significant upregulation of S1R protein expression occurs early in disease pathogenesis and in a region specific manner. Further investigation of regional and temporal S1R expression in Cln6 models, mechanistic analyses of S1R agonist impacts upon ER stress and the unfolded protein response, and analysis of behavioral changes following S1R agonist administration, are ongoing and will help elucidate the therapeutic potential of S1R agonists in CLN6 disease.

S1R agonist treatment reduces pathological hallmarks in *Cln6*^{nclf} primary cortical astrocytes



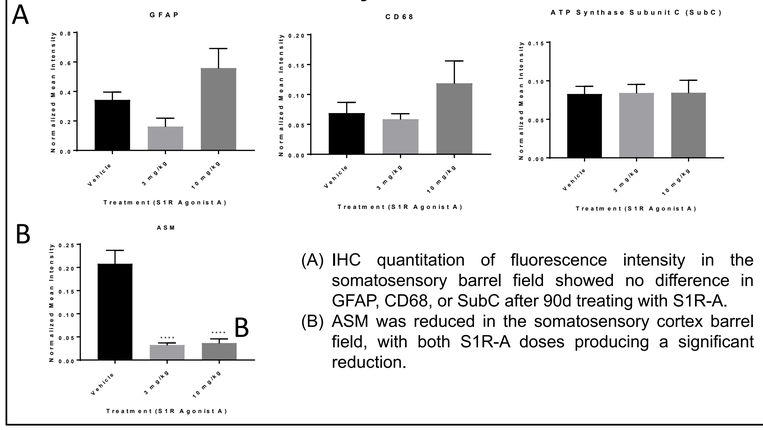
(A) Cln6 astrocyte ICC images representing S1R-A treatment and activation conditions.
(B) ICC quantitation of GFAP fluorescence intensity shows S1R-A treatment reduces astrocytosis in both resting and activated conditions. Subunit C accumulation is reduced in the activated condition for Cln6 cells.
(C) Cln6 astrocyte ICC images representing S1R-B dose response treatment conditions.
(D) ICC quantitation of GFAP fluorescence intensity shows potential dose responsive reductions in astrocytosis.

S1R agonist treatment reduces pathological markers in the VPM/VPL areas of the thalamus in *Cln6*^{nclf} mice



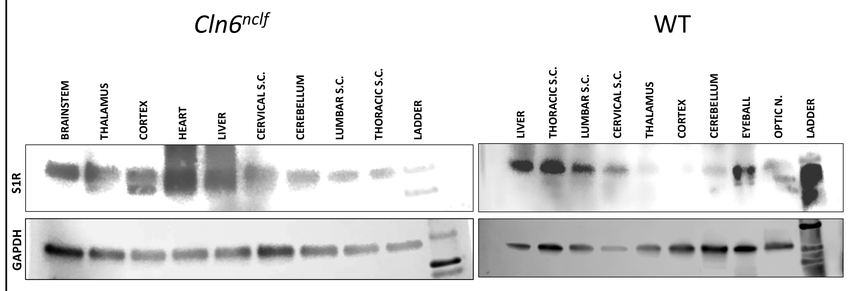
(A) Representative IHC after 90d treatment with S1R-A in P120 *Cln6*^{nclf} mice shows a decrease in ASM and CD68.
(B-D) IHC quantification of fluorescence intensity after 90d treatment show a modest reduction of gliosis marker CD68 and significant decrease in ASM accumulation.

S1R agonist treatment reduces ASM, but not astrogliosis, in the somatosensory cortex of *Cln6*^{nclf} mice



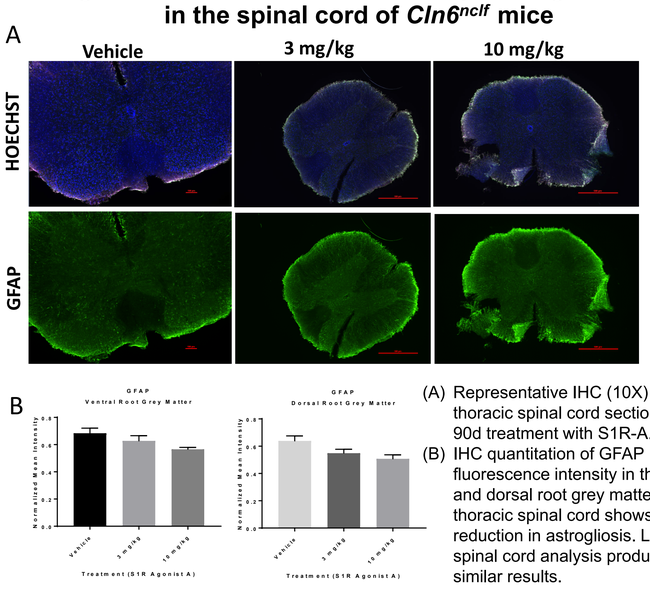
(A) IHC quantitation of fluorescence intensity in the somatosensory barrel field showed no difference in GFAP, CD68, or SubC after 90d treating with S1R-A.
(B) ASM was reduced in the somatosensory cortex barrel field, with both S1R-A doses producing a significant reduction.

S1R protein expression is upregulated in specific neural tissues of 6-7 mo *Cln6*^{nclf} mice compared to wild-type



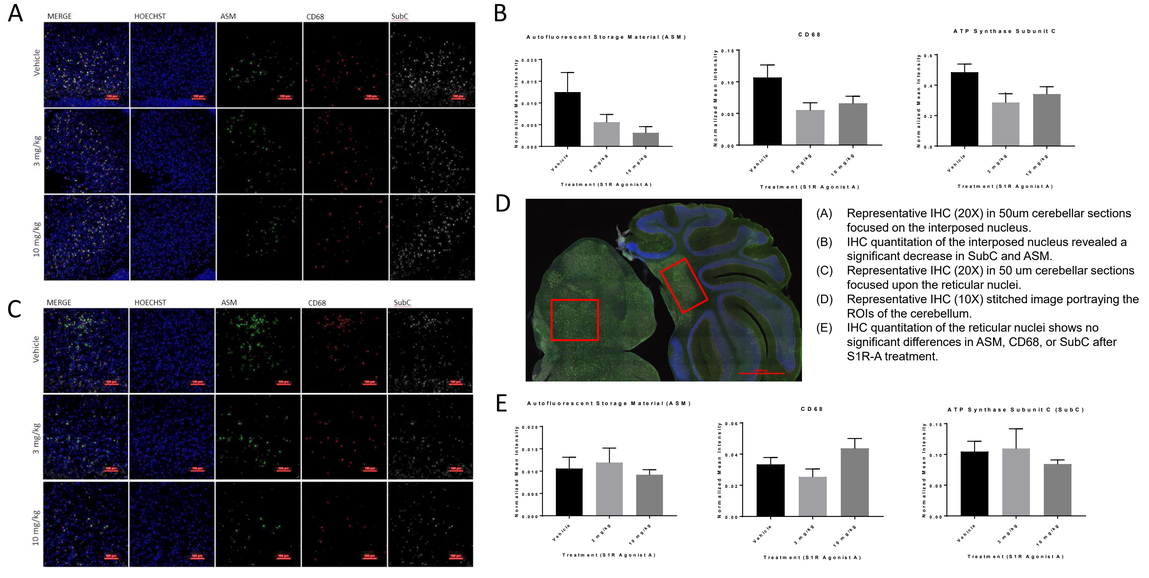
Western blot analysis and comparison of S1R expression across various tissues in *Cln6*^{nclf} and WT mice suggest S1R expression is increased in a region-specific manner, including the liver, thalamus, cortex, and cervical spinal cord.

S1R agonist treatment does not affect pathological markers in the spinal cord of *Cln6*^{nclf} mice



(A) Representative IHC (10X) in 50um thoracic spinal cord sections after 90d treatment with S1R-A.
(B) IHC quantitation of GFAP fluorescence intensity in the ventral and dorsal root grey matter of the thoracic spinal cord shows no reduction in astrogliosis. Lumbar spinal cord analysis produced similar results.

S1R agonist treatment reduces pathological markers in deep cerebellar nuclei of *Cln6*^{nclf} mice



(A) Representative IHC (20X) in 50um cerebellar sections focused on the interposed nucleus.
(B) IHC quantitation of the interposed nucleus revealed a significant decrease in SubC and ASM.
(C) Representative IHC (20X) in 50 um cerebellar sections focused upon the reticular nuclei.
(D) Representative IHC (10X) stitched image portraying the ROIs of the cerebellum.
(E) IHC quantitation of the reticular nuclei shows no significant differences in ASM, CD68, or SubC after S1R-A treatment.

Conclusions and future directions

This study has identified a novel role for S1R activity in the pathogenesis of CLN6 Batten disease. Ongoing work on this project is more broadly evaluating the tissue and cell type specific impacts of S1R activity *in vivo* in CLN6 disease, examining the cellular processes that could be impacted downstream of S1R to define cellular mechanisms at play, detailing temporal changes in S1R over the course of disease progression, and determining if pharmacological targeting of S1R positively impacts neurological deficits in our *Cln6*^{nclf} mice. Ongoing studies will help clarify the role of S1R activity in CLN6 disease and may lead to broader studies of S1R in other NCL variants or interventional studies in CLN6 subjects.

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https://www.curebatten.org
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Charlotte + Gwenyth Gray Foundation
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