

Fine Motor Performance, Brain Volumetry and Metabolism in *Cln2^{R207X/R207X}* Nonsense Point Mutation Model for CLN2 Batten Disease

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1 INTRODUCTION

The *Cln2^{R207X/R207X}* nonsense point mutation mouse model for CLN2 Batten Disease was recently developed to facilitate the development of mutation guided therapies (Geraets RD *et al.* Plos One 2017). This model presents with brain gliosis, lysosomal accumulation of autofluorescent storage material and mitochondrial ATP synthase unit c, and significantly decreased activity of the *Cln2* protein product Tripeptidyl peptidase 1 (TPP1).

The behavioral phenotype of the *Cln2^{R207X/R207X}* model has been shown to involve hyperactivity, tremors, and deficits in the coordinated motor tasks. However, brain structural volumetry, metabolic parameters, and fine motor performance have not been characterized. To strengthen the phenotypic description, we studied *Cln2^{R207X/R207X}* mice for their performance in highly sensitive motor gait analysis, brain volumetry by T2-MRI and neurometabolites of prefrontal cortex (PFC) using proton spectroscopy (¹H-MRS). Data were collected from *Cln2^{R207X/R207X}* and WT mice at 2 and 3 months of age, until humane end point criteria were met.

2 METHODS

All animal experiments were carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by the National Animal Experiment Board, Finland. *Cln2^{R207X/R207X}* and WT littermate mice (n=12/genotype, 6 m + 6 f) at starting age of 2 months were used. Animals were housed at a standard temperature (21 ± 1 °C) and in a light-controlled environment (lights on from 7 am to 8 pm) with ad libitum access to food and water.

Open Field Test: Mice were placed in the center of the chamber and their behavior was recorded for 20 min in 5-minute bins (Med Associates Inc., St Albans, VT, USA). Experiments were conducted during the diurnal phase of the mice, at approximately 10-30 lux of red light.

Fine Motor Kinematic Analysis: The mice were evaluated using an apparatus (Motorater, TSE-systems GmbH, Bad Homburg, Germany) designed for the detection of fine motor skills in rodents. The equipment consists of a brightly illuminated plexiglas corridor (153 x 5 x 10 cm) under which is situated a high-speed camera. The performance of the mice was assessed during walking along the corridor and were recorded with a high speed video-camera (300 fps). The gait and fine motor skills were analyzed from three dimensions (from below and both sides), first using the Simi Reality Motion Systems (Unterschleissheim, Germany) and the obtained raw data was further analyzed by a custom analysis system.

T2-MRI & ¹H-MR Spectroscopy: A horizontal 11.7T magnet was used (Bruker Biospin GmbH, Ettlingen, Germany). Turbo-RARE with in-plane resolution of 78 microns and thirty-one 0.45 mm slices. A voxel was placed in the prefrontal cortex and data was acquired using PRESS sequence (TE/TR = 10/2000 ms).

3 RESULTS

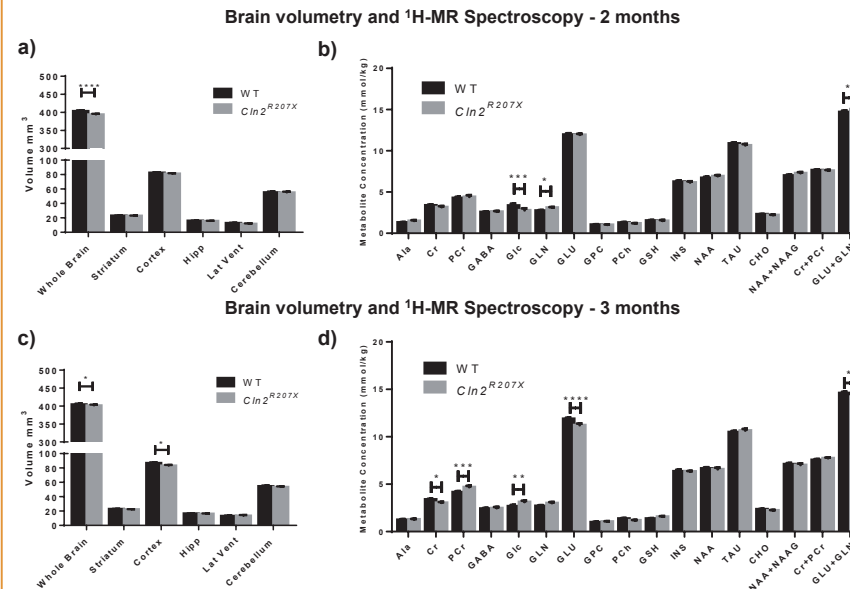


Figure 1. T2-MRI of 2 and 3 month old WT and *Cln2^{R207X}* mice a) and c) respectively, shows a significant decrease in whole brain volume at both time points as well as a decrease in cortical volume at 3-months. Prefrontal cortex ¹H-MRS revealed significant changes in glucose, glutamine, and glutamate + glutamine at 2 months, b). At 3-months, results show significant changes in creatine, phosphocreatinine, glucose, glutamate, and glutamate + glutamine, d).

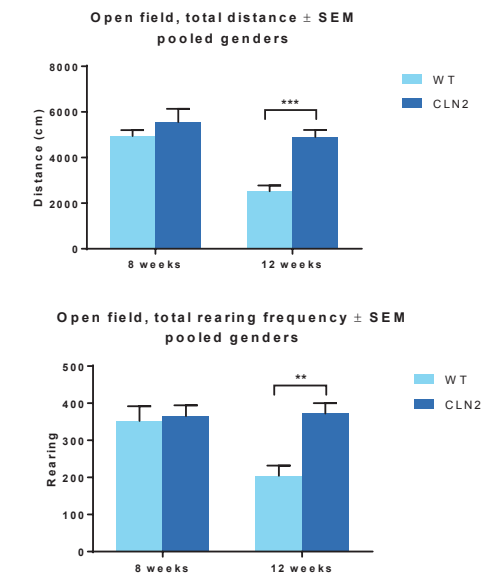


Figure 2. Open field of 3 month old *Cln2^{R207X}* mice displayed an increased distance-traveled, which is associated with hyperactivity as well as increased rearing, which can be a measure of anxiety-like behavior. No differences were observed at 2 months. Data is mean ± SEM, n = 12/genotype.

3 RESULTS CONT'D

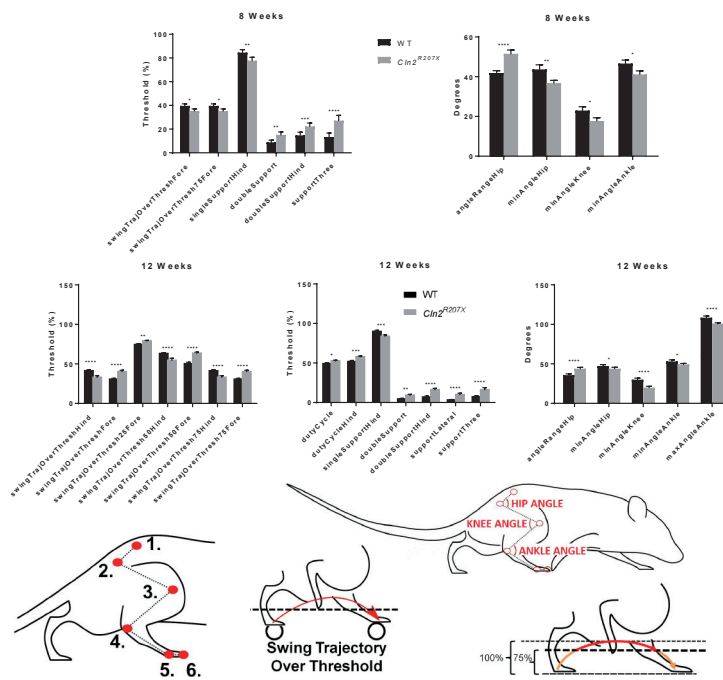


Figure 3. Kinematic gait analysis, comprised of detailed analysis of gait cycle, body and head orientation and positioning during the gait cycle and multiple fore- and hind-limb motion parameters was performed on pooled genders of WT and *Cln2^{R207X}* mice at 2 and 3 months of age. Results show significant differences in a variety of gait factors related to limb movement, flexion, positioning, and support, as well as tail and hip movement.

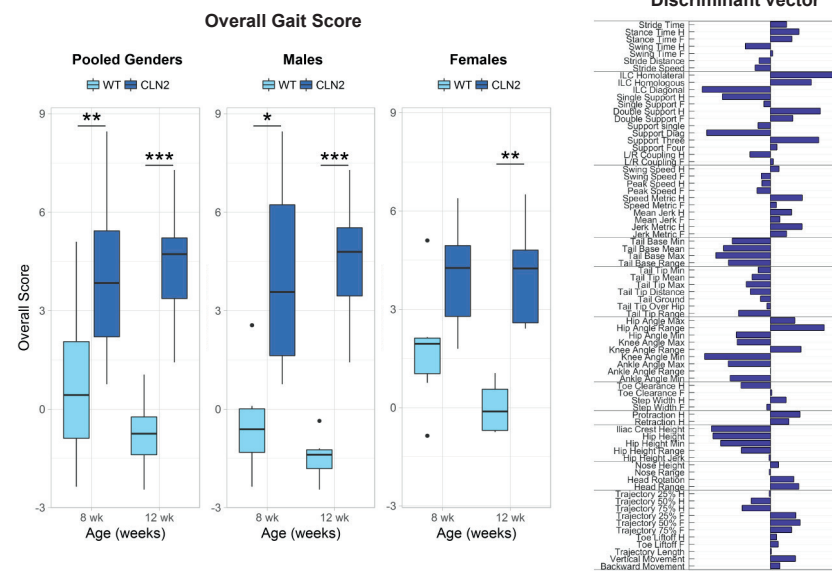


Figure 4. Overall Gait Score, composed of 76 gait variables using PCA, demonstrates highly significant *Cln2^{R207X}* phenotype for pooled genders at both time points. The score difference is significant in males at both time points. In females, the difference is significant at 3 months of age. Data is shown as boxplots. Unpaired Student's t-test, *p<0.05, **p<0.01, ***p<0.001. Influences of each individual parameter to the overall score are presented on the bar graph. There are several characteristic gait features differentiating *Cln2^{R207X}* from the wild type: Changes in the interlimb coordination (ILC) are seen as decreased diagonal gait mode (trotting) and increased double support. Overall hind body posture is lower which can be seen in tail base and hip height parameters, as well as decrease in knee and ankle angles. However, range of hip angle movement is increased. Forelimb swing trajectory profile is elevated, and protraction/retraction is towards head.

4 CONCLUSIONS

- T2-MRI shows a significant decrease in whole brain volume in *Cln2^{R207X}* mice at 2 and 3 months of age as well as a decrease in cortical volume at 3 months.
- ¹H-MRS results display significant changes in a variety of metabolite concentrations in *Cln2^{R207X}* mice at 2 and 3 months.
- Open field behavior testing increases in distance-traveled and rearing behavior by *Cln2^{R207X}* mice at 3-months old.
- Cln2^{R207X}* mice exhibit significant differences in kinematic gait measurements at 2 and 3 months of age.
- Principal Component Analysis on kinematic gait analysis data compressed the changes to overall scores of *Cln2^{R207X}* mice and shows significant difference at 2 and 3 months.
- Future work will be focused on determining any brain region-specific white matter or metabolism deficits via diffusion tensor imaging (DTI) and ¹⁸F-fluorodeoxyglucose positron emission tomography, respectively.