**Title**: A Longitudinal Investigation of Auditory Evoked Potentials (AEPs) in CLN3 Batten Disease

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**Introduction**: CLN3 is one of the most common forms of Batten Disease, a rare lysosomal storage disorder. Symptom onset typically occurs between 4-7 years old[1,2] beginning with vision loss that progresses into blindness followed by progressive cognitive decline, seizures, and Parkinsonism [1-4]. Due to the nature of symptom progression and severity, it is challenging to accurately measure the cognitive abilities of patients with CLN3 Batten disease, specifically in later stages where they have severe deficits in verbal communication abilities[5,6]. Little is known about sensory processing abilities across stages of CLN3 Batten disease. This work investigates longitudinal changes in early auditory processing in patients with CLN3 Batten disease using electroencephalography (EEG) and event related potential (ERP) techniques. The goal of this work is to identify objective biomarkers that can be used to inform disease progression and track treatment response[7].

**Method**: Four participants with CLN3 Batten disease and 49 typically developing (TD) controls completed an auditory mismatch negativity paradigm (aMMN). The paradigm involves participants listening to a series of regularly occurring standard tones (85%) interspersed with deviant tones (15%). Participants completed three different conditions of the aMMN task which altered the stimulus onset asynchrony (SOA) between tones (450ms, 900ms, or 1800ms). Standard tones were 100ms, 1000Hz while deviant tones were 180ms, 1000Hz. Participants with CLN3 Batten disease completed this task across 4 to 6 timepoints (each timepoint was at least 6 months apart) and clinical data, including the Unified Batten Disease Rating Scale (UBDRS), and CLN3 Staging System (CLN3SS) were collected at each visit. The CLN3SS separates patients into four distinct stages from 0 (least severe) to 3 (most severe). The latter was used to classify disease progression at each timepoint[8,9]. Across time points, participant 1 ranged from 12-18 years, participant 2 ranged from 11-15 years, participant 3 ranged from 19-25 years, and participant 4 ranged from 19-25 years old. TD control participants completed this task once and were split by age into two comparison groups ages 9-18 (n=27), and 19-30 (n=22). We examined the grand average ERPs from these TD control groups and compared them with ERPs from the CLN3 participants at each timepoint. Data were collected using both 32 channel and 64 channel electrode arrays. For this analysis, the auditory evoked potential (AEP) from standard tones was analysed. Standard tones from all conditions at each visit were averaged together to create a robust AEP. We examined the P1, N1, P2, and N2 which show early sensory processing, auditory attention, higher level stimulus evaluation and conflict monitoring, respectively.

**Results**: Both TD control groups show small AEP amplitudes, with a P1 occurring around 80-90ms post stimulus, the N1 occurring around 100-110ms post stimulus, and the P2 occurring between 150-165ms post stimulus. The N2 occurred about 250ms post stimulus in the 9-18 year old controls and almost no N2 was present in the 19-30 year old controls. CLN3 Participants 1 and 2 showed a similar pattern in their AEPs; both show a gradually decreasing amplitude of their N2 components. Participant 1 progressed from CLN3SS 1 in timepoint 1 to 2 for timepoints 2-4. Participant 2 was at CLN3SS 2 for all timepoints. Participant 3 showed an interesting reduction in P2 amplitude and a diminished decreasing amplitude of the N2 component over time. Participant 3 had an CLN3SS of 2 for timepoints 1-3 and progressed to a CLN3SS 3 at timepoint 4. The AEPs for patients 1-3 show a similar pattern to that of controls but with increased variability in amplitude. Participant 4 shows a positive peak at approximately 300ms which is not present in control data, this peak stabilizes across visits. The overall pattern of the AEP in participant 4 is consistent across time, but highly differentiated from control data. Participant 4 had a CLN3SS of 2 at time 1, and 3 for all subsequent timepoints.

**Discussion:** Substantial changes in the AEP are seen in participants with CLN3 Batten disease across time despite limited change in their CLN3SS, indicating that changes in neural processing may be occurring. Additionally, the AEPs are differentiated between patients which is consistent with the heterogeneity of clinical presentations and disease progression seen in CLN3 Batten disease. Overall, the AEP is an easy to collect, robust, and well-studied ERP. This study demonstrates that the AEP is sensitive to changes over time and has potential to be used as an objective biomarker to track disease severity and potential treatment progress in future clinical trials for CLN3. It will be important to continue to identify individual differences in the AEPs in patients with CLN3 Batten disease and future work should focus on patients as individuals to accurately characterize changes in the AEP over time in CLN3 patients.

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