Preliminary Results on Long-Term Potentiation-Like Cortical Plasticity and Cholinergic Dysfunction After Miglustat Treatment in Niemann-Pick Disease Type C

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Abstract Niemann-Pick disease type C (NPC) is a rare autosomal recessive lysosomal storage disorder, which manifests clinically with a wide range of neurological signs and symptoms. We assessed multiple neurological, neuropsychological and neurophysiological biomarkers using a transcranial magnetic stimulation (TMS) multi-paradigm approach in two patients with NPC carrying a homozygous mutation in the *NPC1* gene, and in two heterozygous family members.

We assessed short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), long-interval intracortical inhibition (LICI), short-latency afferent inhibition (SAI) and long-term potentiation (LTP)-like cortical plasticity with a paired associative stimulation (PAS) protocol.

Baseline SAI and LTP-like plasticity were impaired in both patients with NPC and in the symptomatic heterozygous *NPC1* gene mutation carrier. Only a limited decrease in SICI and ICF was observed, while LICI was within normal

range in all subjects at baseline. After 12 months of treatment with miglustat, a considerable improvement in SAI and LTP-like plasticity was observed in both patients with NPC. In conclusion, these biomarkers could help to confirm the diagnosis of NPC, and may give an indication of prognostic outcomes in pharmacological trials.

Introduction

Niemann-Pick disease type C (NPC) is a rare genetic disorder, which results in a wide range of neurological, visceral and psychiatric signs and symptoms, including cerebellar ataxia, cataplexy, seizures and dementia (Vanier 2010). This autosomal recessive lysosomal storage disorder (LSD) is caused by mutations of either the *NPC1* gene (in 95% of families) (Carstea et al. 1997) or the *NPC2* gene (Naureckiene et al. 2000). The incidence of NPC is estimated to be one in every 100,000 live births, although the late-onset phenotypes or variant forms may have a much higher incidence, ranging from 1:19,000 to 1:36,000 (Wassif et al. 2015).

Although the exact functions of the proteins derived from the *NPC1* and *NPC2* genes are still to be fully elucidated, the disease is characterized by clear abnormalities in intracellular transport of endocytosed cholesterol with sequestration of unesterified cholesterol in lysosomes and late endosomes, resulting in downstream effects on cholesterol homeostasis (Pentchev et al. 1985; Liscum et al. 1989). NPC is also characterized by the accumulation of beta amyloid (Yamazaki et al. 2001) and neurofibrillary tangles (Love et al. 1995), which are immunologically and ultrastructurally

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similar to those seen in Alzheimer's disease (AD) (Auer et al. 1995). The similarities between NPC and AD extend to the cholesterol pathway, which has been repeatedly implicated in the pathogenesis of AD (Liu et al. 2013). This commonality between the two conditions is substantiated by observations that cholesterol levels can affect the processing of amyloid precursor protein (Bodovitz and Klein 1996) and accumulation of beta amyloid peptides (Simons et al. 1998), and is further supported by the notion that the $\epsilon 4$ isoform of apolipoprotein E promotes disease progression in both disorders (Corder et al. 1993; Fu et al. 2012).

Recently, abnormalities in cortical plasticity have been demonstrated in AD using non-invasive brain stimulation techniques, including transcranial magnetic stimulation (TMS) (Di Lorenzo et al. 2016). These studies were based on the strong evidence obtained from electrophysiological recordings in animal models of AD, which highlighted an impairment in hippocampal long-term potentiation (LTP) mediated by beta amyloid peptides and tau proteins (Palop and Mucke 2010). *Npc1*-mutant mice also show impaired hippocampal synaptic plasticity (D'Arcangelo et al. 2016), although in vivo evidence for impairment in LTP-like cortical plasticity in patients with NPC is currently lacking. In the most recent of these studies, miglustat (Zavesca[®], Actelion Pharmaceuticals, Allschwil, Switzerland), a small iminosugar molecule that reversibly inhibits glycosphingolipid synthesis, was shown to rescue deficits in synaptic plasticity in *Npc1*-mutant mice (D'Arcangelo et al. 2016). Furthermore, an impairment in short-latency afferent inhibition (SAI), a measurement of sensorimotor integration obtained from TMS, has recently been observed in NPC, further supporting the link between NPC and AD (Manganelli et al. 2014). SAI is thought to be partially mediated by central cholinergic transmission; the restoration of SAI with the use of acetylcholinesterase inhibitors supports this hypothesis, and seems to correlate with the response to treatment (Di Lazzaro et al. 2005).

The aim of this study was to evaluate specific neurophysiological parameters, including SAI and LTP-like cortical plasticity, as well as short-interval intracortical inhibition (SICI), intracortical facilitation (ICF) and long-interval intracortical inhibition (LICI), in two previously described monozygotic twins homozygous for *NPC1* mutations (Benussi et al. 2015a), compared with those in two heterozygous family members and 18 healthy controls. SICI, ICF, LICI and SAI are TMS paired-pulse protocols which reflect specific intracortical circuits; SICI has been shown to reflect intracortical post-synaptic inhibition mediated by GABA_A receptors, ICF to reflect excitatory glutamatergic circuits while LICI to be mediated by GABA_B receptors (Benussi et al. 2015b).

Subjects heterozygous for *NPC1* mutations were evaluated because these individuals may exhibit neurophysio-

logical manifestations of the signs and/or symptoms of NPC at a subclinical level (Josephs et al. 2004; Kluene-mann et al. 2013). We further aimed to assess the effects of 12 months of treatment with miglustat on neurophysiological parameters in the two patients with overt NPC.

Materials and Methods

Subjects

Two monozygotic twins bearing a homozygous pathogenic mutation of *NPC1* (Patient 1 [P1] and Patient 2 [P2]) participated in the present study (Benussi et al. 2015a). One brother (Patient 3 [P3]) and the mother of the twins (Patient 4 [P4]), who were heterozygous for the *NPC1* mutation, as well as 18 healthy controls, were also included. P1 and P2 were evaluated before and after 12 months of treatment with miglustat.

Each subject underwent an extensive clinical work-up and neurological examination, as well as eye movement assessment (EyeSeeCam video-oculography [VOG] system, with the camera placed over the left eye).

Transcranial Magnetic Stimulation

TMS was performed with a figure-of-eight coil (each loop was 70 mm in diameter) connected to a Magstim BiStim² system (Magstim Company, Oxford, UK). Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle (FDI) via surface Ag/AgCl electrodes placed in a belly-tendon montage and recorded using a BIOPAC MP150 electromyograph (BIOPAC Systems Inc., Santa Barbara, CA, USA).

rMT was defined as the minimal stimulus intensity needed to produce MEPs with an amplitude of at least 50 μ V in 5 out of 10 consecutive trials during complete muscle relaxation. This was controlled by visually checking the absence of electromyography (EMG) activity at high-gain amplification.

SICI-ICF, LICI and SAI were studied at rest via a paired-pulse paradigm, delivered in a conditioning-test design, as previously reported (Benussi et al. 2016). For SICI-ICF, the conditioning stimulus was set at an intensity of 70% of the rMT, and the test stimulus was adjusted to evoke an MEP approximately 1 mV peak-to-peak in the relaxed FDI. Inter-stimulus intervals (ISIs) between the conditioning stimulus and the test stimulus of 1, 2, 3 and 5 ms were employed to investigate SICI, and ISIs of 7, 10 and 15 ms were used to investigate ICF.

LICI was elicited by applying two suprathreshold stimuli at long ISIs (50, 100 and 150 ms), with the conditioning stimulus set at 130% of the rMT preceding the test

stimulus, adjusted to evoke an MEP of approximately 1 mV peak-to-peak.

For SAI, the conditioning stimuli were single 200 μ s pulses of electrical stimulation applied to the right median nerve at the wrist through bipolar electrodes (cathode proximal). The intensity of the conditioning stimulus was set at just over the threshold for evoking a visible twitch of the thenar muscles, and the test stimulus was adjusted to evoke an MEP of approximately 1 mV peak-to-peak. ISIs were determined relative to the N20 component of the somatosensory evoked potential induced by stimulation of the right median nerve. The conditioning stimulus preceded the test stimulus by ISIs of -4 , 0 , $+4$ and $+8$ ms.

In all participants, 20 stimuli were delivered in a pseudorandomized sequence for each ISI, and for the test stimulus alone, for all stimulation paradigms (SICI-ICF, LICI and SAI). The amplitude of the conditioning MEPs was expressed as a ratio of the mean unconditioned response. The inter-trial interval was set at 5 s ($\pm 10\%$).

LTP-like effects were probed using a paired associative stimulation (PAS) protocol. PAS consisted of 200 electrical stimuli to the median nerve, paired with TMS stimuli over the contralateral motor cortex given at 0.25 Hz. The median nerve was stimulated at the wrist with standard bar electrodes (cathode proximally); pulses were constant current square waves with a width of 200 μ s at an intensity of 300% of the perceptual threshold. Each TMS stimulus was adjusted to 1 mV MEP intensity and was preceded by the electrical conditioning stimulus at an ISI of 25 ms. Using an average of 30 responses, the mean MEP amplitude was determined at baseline and at 0, 10, 20 and 30 min after PAS from the right abductor pollicis brevis (APB) muscle, using the same stimulator output at all time points.

Complete muscle relaxation was monitored by audiovisual feedback throughout the experiment, where appropriate. If the quality of the data was degraded by movement of the patient, the protocol was recommenced and the initial data discarded.

Results

Two homozygous and two heterozygous *NPC1* mutation carriers, as well as 18 healthy controls were included in the study. Demographic and clinical characteristic for included patients are reported in Table 1.

Patient 1

P1 was a 25-year-old male monozygotic twin with moderate neurological impairment, including ataxic gait, limb dysmetria and supranuclear vertical gaze ophthalmoparesis, associated with splenomegaly from birth. Neuro-

psychological evaluation revealed global cognitive deficits, in particular in short- and long-term verbal memory, working memory and executive functions. Assessment of eye movements, using the EyeSeeCam VOG system over the left eye, revealed very slow and small vertical saccades (downward more than upward), mildly slowed horizontal saccades, and low-gain smooth pursuit, especially in the vertical plane. Molecular analysis revealed a homozygous c.2662 C>T (p.P888S) mutation in the *NPC1* gene (Benussi et al. 2015a).

At baseline, before initiating treatment with miglustat, neurophysiological analysis with TMS revealed a clear impairment in SAI (mean 87.4% at ISI 0 and +4) and LTP-like plasticity (mean 116.1% after PAS protocol) compared with healthy controls. Furthermore, a limited impairment in SICI (mean 55.1% at ISI 1, 2, 3 and 5 ms) and ICF (mean 124.4% at ISI 7, 10 and 15 ms) was observed, while LICI (mean 31.4% at ISI 50, 100 and 150 ms) was similar to healthy controls (see Table 1 and Fig. 1).

Patient 2

P2 was the monozygotic twin of P1. Unlike his twin brother, he presented with only mild neurological and neuropsychological impairment, as well as splenomegaly. VOG revealed moderately slowed downward saccades, whereas upward saccades were minimally slowed, and horizontal saccades were normal, as was vertical and horizontal smooth pursuit.

As with P1, a homozygous c.2662 C>T (p.P888S) mutation in the *NPC1* gene was detected (Benussi et al. 2015a).

Neurophysiological analysis at baseline revealed a clear impairment in SAI (mean 81.4%) and LTP-like plasticity (mean 133.4%) compared with healthy controls but to a lesser extent than was observed in P1. SICI (mean 43.5%) and LICI (mean 27.0%) were within normal range, while ICF (mean 126.8%) results were similar to those of P1 (see Table 1 and Fig. 1).

Patient 3

P3 was the 34-year-old non-twin brother of P1 and P2. He was asymptomatic and the neurological examination was unremarkable. Eye movement assessment with VOG was also unremarkable. Molecular analysis revealed a heterozygous c.2662 C>T (p.P888S) mutation in the *NPC1* gene.

Neurophysiological analysis revealed only a very mild impairment in SAI (mean 60.9%) and LTP-like cortical plasticity (mean 146.9%) compared with healthy controls, while SICI (mean 37.2%), ICF (mean 145.8%) and LICI (mean 28.9%) were within normal range (see Table 1 and Fig. 1).

Table 1 Demographic and neurophysiological characteristics of enrolled patients

	HC (<i>n</i> = 18)	P1	P2	P3	P4
Age (years)	39.2 ± 15.7 (52.6)	25	25	34	57
rMT (%)	45.5 ± 7.1	40.0	47.0	44.0	45.0
Mean SICI 1, 2, 3, 5 ms (%)	33.6 ± 3.6	55.1	43.5	37.2	40.2
Mean ICF 7, 10, 15 ms (%)	152.0 ± 6.4	124.4	126.8	145.8	118.8
Mean LICI 50, 100, 150 ms (%)	26.5 ± 3.6	31.4	27.0	28.9	30.3
Mean SAI 0, 4 ms (% at baseline)	49.5 ± 23.7	87.4	81.4	60.9	75.5
Mean SAI 0, 4 ms (% after treatment)	—	70.8	60.3	—	—
LTP-like plasticity (% at baseline)	161.9 ± 32.3	116.1	133.4	146.9	127.5
LTP-like plasticity (% after treatment)	—	136.8	150.1	—	—

Demographic, clinical and neurophysiological characteristics are expressed as mean ± standard deviation, and (range) for age; rMT is expressed as percentage of the maximum stimulator output; SICI, ICF, LICI and SAI are expressed as percentage of mean MEP amplitude related to the control MEP; LTP-like plasticity is expressed as change (%) in mean MEP amplitude at 0, 10, 20 and 30 min after PAS protocol

HC healthy controls, ICF intracortical facilitation, LICI long-interval intracortical inhibition, LTP long-term potentiation, MEP motor evoked potential, rMT resting motor threshold, SAI short-latency afferent inhibition, SICI short-interval intracortical inhibition

Fig. 1 SICI-ICF, LICI, SAI and LTP-like cortical plasticity in patients and HCs. **(a)** SICI-ICF, **(b)** LICI and **(c)** SAI at different ISIs. Data are plotted as a ratio to the unconditioned MEP amplitude. **(d)** PAS effect on corticospinal excitability, as measured by change in 1 mV MEP amplitude at various time points. Error bars represent standard errors.

HC healthy controls, ICF intracortical facilitation, ISI inter-stimulus interval, LICI long-interval intracortical inhibition, LTP long-term potentiation, MEP motor evoked potential, P1/2/3/4 Patient 1/2/3/4, PAS paired associative stimulation, Pre pre-PAS baseline, SAI short-latency afferent inhibition, SICI short-interval intracortical inhibition

Patient 4

P4 was the 57-year-old mother of P1, P2 and P3, and was initially evaluated at the Neurology ward of the University of Brescia Hospital (Brescia, Italy) for a resting tremor in her right arm. She presented with a history of mild spleen enlargement (bipolar diameter of 12 cm) for which numerous investigations were performed; all were inconclusive.

Neurological examination revealed an asymmetric extrapyramidal syndrome with a moderate rest tremor, bradykinesia and increased rigidity in the right upper limb, with a Unified Parkinson's Disease Rating Scale – part III (UPDRS-III) score of 15/108. No deficits in cranial nerves were detected and ocular movements were within normal range, although a slight dysmetria in ocular movements was detected with VOG. The patient did not report any Parkinsonian non-motor symptoms, such as hyposmia, constipation, orthostatic hypotension or rapid eye movement sleep behaviour disorder.

Routine laboratory evaluations, including free iron, ferritin, transferrin, free copper and ceruloplasmin levels, were within normal range.

Brain magnetic resonance imaging results were unremarkable, while ^{123}I -FP-CIT (DaTScan) single-photon emission computed tomography imaging revealed a symmetric decreased uptake in the striatum, with a greater impairment in the putamen.

Treatment with levodopa (levodopa/carbidopa 100 mg/25 mg 3 times daily for 1 week) ameliorated extrapyramidal signs and symptoms, with a significant improvement in the UPDRS-III score (from 15/108 to 10/108).

Molecular analysis revealed a heterozygous c.2662 C>T (p.P888S) mutation in the *NPC1* gene, while mRNA analysis with reverse transcription polymerase chain reaction, and copy number analysis of the *NPC1* and *NPC2* genes with multiplex ligation-probe amplification, did not identify an impairment in mRNA processing or in the number of copies of the analysed genes, respectively. Filipin test showed accumulation of unesterified cholesterol in perinuclear vesicles consistent with a “variant phenotype”. Plasmatic oxysterols were significantly increased: cholestane-3 β -5 α -6 β -triol of 42.35 ng/ml (normal values <20.45 ng/ml) and 7-ketocholesterol 109.92 ng/ml (normal values <45.58 ng/ml).

Neurophysiological analysis revealed a clear impairment in ICF (mean 118.8%), SAI (mean 75.5%) and LTP-like cortical plasticity (mean 127.5%) compared with healthy controls, while SICI (mean 40.2%) and LICI (mean 30.3%) were within normal range (see Table 1 and Fig. 1).

Effect of Miglustat Treatment on Neurophysiological Parameters

After baseline, neurological, neuropsychological and neurophysiological evaluation, P1 and P2 were initiated on miglustat treatment and were re-evaluated after 12 months. No notable side effects were reported except for gastrointestinal disturbances and diarrhea in P1.

Both subjects reported an improvement in neurological symptoms; in particular, P1 regained the ability to climb stairs unassisted and his gait visibly improved. P1 also reported a decrease in recurring intrusive thoughts that caused him significant distress and anxiety, which, in the past, he had tried to suppress with repetitive motor behaviors.

On neurological examination, clear improvement in dysarthria, ataxia, gait speed and balance, limb dysmetria, postural tremor and dysidiadochokinesia was observed in P1, while P2 displayed only a mild supranuclear vertical gaze ophthalmoparesis, only on downgaze, similar to pre-treatment conditions. Neuropsychological evaluation revealed a definite improvement in executive functions, calculation ability, and semantic and phonemic fluency in P1, while P2 showed an improvement in semantic and phonemic fluency and visuospatial abilities (see Table 2).

Neurophysiological evaluation after 12 months of treatment with miglustat revealed a considerable improvement in SAI and LTP-like plasticity in both P1 and P2 compared with baseline (see Table 1 and Fig. 2; for a scatterplot distribution of single patients regarding SAI and PAS see Supplementary Figure). No significant differences were observed in RMT and 1 mv MEP thresholds at 12 months.

Discussion

This study provides novel in vivo evidence of a substantial impairment in LTP-like cortical plasticity in symptomatic patients bearing homozygous mutations in the *NPC1* gene, and to a limited extent in heterozygous *NPC1* gene mutation carriers, when compared with healthy controls. Our findings also confirm and extend a previous report of impaired SAI in patients with NPC (Manganelli et al. 2014).

Numerous studies have highlighted impairment in LTP-like cortical plasticity in neurodegenerative disorders, including in AD and frontotemporal dementia (Di Lorenzo et al. 2016; Benussi et al. 2016). Using a PAS protocol that has previously been shown to induce LTP-like effects in healthy subjects (Wolters 2003), our study demonstrates

Table 2 Neuropsychological assessment after 12 months of miglustat treatment

Test	P1 Before treatment ^a	P1 Follow-up	P2 Before treatment ^a	P2 Follow-up
MMSE	22	27	30	30
MOCA	21	25	25	25
Babcock story recall test	4	4	10.5	10.5
Forward digit span	3.75	5.75	4.25	4.25
Backward digit span	3.42	2.42	3.42	3.42
Semantic fluency	20	33	30	36
Phonemic fluency	11	17	17	25
Clock drawing	10	10	10	10
Rey figure copy	29.5	30.5	22	29.5
Rey figure recall	10.75	13.75	11.5	16.5
Trial making A (s)	116	104	52	47
Trial making B (s)	Np	261	163	160
Raven's progressive matrices	33.75	32	35	35

Pathological scores are in boldface according to Italian normative data

MMSE Mini-Mental State Examination, MOCA Montreal Cognitive Assessment, Np not able to perform, P1/2 Patient 1/2

^aData previously published (Benussi et al. 2015a)

Fig. 2 Effect of miglustat treatment on SAI and LTP-like cortical plasticity. **(a)** SAI at different ISIs in P1 and P2 at baseline and after 12 months of miglustat treatment, and in HCs. Data are plotted as a ratio to the unconditioned MEP amplitude. **(b)** PAS effect on corticospinal excitability, as measured by change in 1 mV MEP

amplitude at various time points, at baseline and after 12 months of miglustat treatment. Error bars represent standard errors. HC healthy controls, ISI inter-stimulus interval, MEP motor evoked potential, P1/2 Patient 1/2, PAS paired associative stimulation, SAI short-latency afferent inhibition

lower motor cortex excitability after PAS in patients with mutations of the *NPC1* gene compared with healthy controls. The impairment in LTP-like synaptic plasticity

observed in patients with NPC is supported by the notion that hippocampal LTP is impaired in *Npc1*-mutant mice (D'Arcangelo et al. 2016). On the other hand, only modest

alterations in SICI and ICF were observed in P1, P2 and P4. SICI is thought to reflect short-lasting postsynaptic inhibition, mediated by the γ -aminobutyric acid (GABA)_A receptor at the local interneuron level, while ICF is thought to represent net facilitation of neuronal excitability, most likely mediated by glutamatergic *N*-methyl-D-aspartate (NMDA) receptors (Ziemann et al. 2015). Our findings therefore suggest mild impairments in GABA_A receptor-mediated and NMDA receptor-mediated neurotransmission in patients with *NPC1* gene mutations. Although we did not identify a significant difference in LICI, which is thought to be mediated by GABA_B receptors, generalized axonal dystrophy in GABAergic cell populations is a common finding in neuronal storage disorders (Gibson et al. 2008). Furthermore, defects in synaptic transmission at both GABAergic and glutamatergic synapses have been identified in mouse models of NPC, which may result, at least in part, from an impairment of synaptic vesicle trafficking (Byun et al. 2006). Together, these findings suggest that deficits in LTP-like plasticity and SICI-ICF may be early indicators of NPC, and could provide a potential explanation for key features of the disease, such as dystonia and seizures.

The clear impairments in SAI in this study seem to correlate with disease severity and with mutation status. Manganelli et al. (2014) recently reported similar impairments in SAI in three patients with NPC, providing evidence to support the hypothesis that cholinergic alterations may play a role in cognitive impairment in patients with this disease. This hypothesis is further substantiated by reports that patients with NPC suffer from grey matter atrophy in subcortical nuclei that are implicated in the central cholinergic pathway, including the thalamus, hippocampus and basal forebrain (Walterfang et al. 2011, 2013).

Another important finding in this study was the modest impairment in SAI and LTP-like plasticity in heterozygous family members, particularly in P4, who was symptomatic for Parkinson's disease. Individuals heterozygous for *NPC1* mutations are generally considered to be clinically unaffected, but it is uncertain whether *NPC1* haploinsufficiency can predispose *NPC1* mutation heterozygotes to intermediate and potentially subclinical NPC phenotypes. Biochemically, *NPC1* gene mutation carriers have an intermediate phenotype in terms of lipid regulation and metabolism. It has been reported that cultured skin fibroblasts from individuals heterozygous for *NPC1* gene mutations have an intermediate rate of cholesterol esterification, production of cholesteryl esters, and unesterified cholesterol storage level compared with cells from healthy individuals and those homozygous for *NPC1* gene mutations (Hung et al. 2016). Furthermore, in human *NPC1* gene mutation carriers, the concentrations of plasma oxysterols, 7-keto-

cholesterol and cholestane-3 β -5 α -6 β -triol are significantly elevated compared with those in healthy individuals (Porter et al. 2010). Central nervous system abnormalities in heterozygous *Npc1*-mutant mice are suggestive of neurodegeneration with a significant loss of Purkinje cells, and increase in brain cholesterol and hyperphosphorylated tau (Mattsson et al. 2012). Consistent with these animal studies, case reports have described Parkinsonism or Lewy body pathology in *NPC1* gene mutation carriers (Josephs et al. 2004; Klunemann et al. 2013; Chiba et al. 2014). Our results suggest that the notion that heterozygous *NPC1* gene mutation carriers are clinically unaffected may not always be accurate, and therefore, warrants further investigation. The case here described with a parkinsonian syndrome raises the possibility that NPC could be another storage disease in which mutations are a risk factor for PD. Potential mechanisms similar to the GBA association have been suggested, including the partial loss of enzyme function altering cytoplasmic lipid homeostasis or overwhelming the ubiquitin-proteasome pathway (Klunemann et al. 2013). However, because PD is a relatively common neurologic disease it is possible that such associations are simply a rare coincidence.

The second aim of the present work was to evaluate the neurological, neuropsychological and neurophysiological status of patients after 12 months of treatment with miglustat. Whether or not miglustat can be beneficial for *NPC1* mutation carriers with a biochemical profile compatible with an LSD remains to be confirmed. After 12 months of treatment with miglustat, we observed a clear improvement in dysarthria, gait ataxia, limb dysmetria and dysidiadochokinesia in P1, while P2 remained stable upon neurological examination. Cognitive assessment revealed a clear improvement in calculation ability, semantic and phonemic fluency, and visuospatial abilities. More interestingly, along with clinical improvement, cholinergic impairment seemed to be reduced, and we observed an increase in LTP-like cortical plasticity after treatment with miglustat in both P1 and P2.

We acknowledge that the number of subjects included in this study was very small, owing to the fact that NPC is a rare disorder, and this raises the important issue of how we can generalize these results to other patients with NPC. Furthermore, differences in patients compared to controls could also be accountable to other unknown shared family genetics. Nevertheless, our findings provide novel evidence of reduced LTP-like cortical plasticity in patients with NPC, which seemed to be rescued by treatment with miglustat.

We conclude that impaired SAI and LTP-like plasticity may represent biomarkers that could further support the diagnosis of NPC, and may give an indication of prognostic outcomes in clinical trials.

Author Contributions

Conception and design of the study: AB, AP and BB. Acquisition and analysis of data: AB, MSC, MC, VB, MT, ES, AD, AP and BB. Drafting the manuscript and figures: AB, ES, AP and BB.

Conflicts of Interest

AB, MSC, MC, VB, MT, ES, AD, AP, BB declare that they have no conflict of interest.

Ethical Standards

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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