Niemann-Pick type C disease: Effects of a therapy with acetyl-DL-leucine and vestibular function

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### Abbreviations

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AD</td>
<td>Alzheimer`s disease</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked ImmunoSorbent Assay</td>
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<tr>
<td>[¹⁸F]-FDG-PET</td>
<td>18F-Fluoro-desoxyglucose-PET</td>
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<tr>
<td>FEF</td>
<td>Frontal Eye Field</td>
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<td>GD3</td>
<td>Gaucher disease type 3</td>
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<td>INC</td>
<td>Nucleus interstitialis Cajal</td>
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<td>MVN</td>
<td>Medial vestibular nucleus</td>
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<td>NFT</td>
<td>Neurofibrillary tangles</td>
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<td>NP-C</td>
<td>Niemann-Pick disease type C</td>
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<td>NPH</td>
<td>Nucleus praepositus hypoglossi</td>
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<td>mDRS</td>
<td>modified Disability Rating Scale</td>
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<td>PEF</td>
<td>Parietal Eye Field</td>
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<td>PPRF</td>
<td>Paramedian pontine reticular formation</td>
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<td>rCGMglc</td>
<td>Regional cerebral metabolism for glucose</td>
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<td>riMLF</td>
<td>Rostral interstitial nucleus of medial longitudinal fascicle</td>
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<td>SARA</td>
<td>Scale for the Assessment and Rating of Ataxia</td>
</tr>
<tr>
<td>SCAFI</td>
<td>Spinocerebellar Ataxia Functional Index</td>
</tr>
<tr>
<td>UVL</td>
<td>Unilateral vestibular loss</td>
</tr>
<tr>
<td>VC</td>
<td>Vestibular compensation</td>
</tr>
<tr>
<td>VSGP</td>
<td>Vertical supranuclear gaze palsy</td>
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<td>VSSP</td>
<td>Vertical supranuclear saccade palsy</td>
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Summary

Niemann-Pick type C (NP-C) is an autosomal recessive lysosomal storage disorder. Symptoms of NP-C disease are heterogeneous and include neurologic, psychiatric and systemic manifestations. The leading symptom of the disease is cerebellar ataxia, which considerably reduces the quality of life and functioning of these patients.

There is no causal cure and progressive Purkinje cell degeneration leads to severe ataxia. There is a disease-specific therapy with miglustat, a small iminosugar molecule that reversibly inhibits glycosphingolipid synthesis, reducing the progression rate of the disease. Thus, symptomatic therapies of ataxia are needed to alleviate the burden of the disease and improve the quality of life of patients and their caregivers.

This dissertation includes two studies that were concerned with the treatment and pathophysiology of NP-C disease. The first study evaluates the effects of the modified amino-acid acetyl-DL-leucine in a cohort of twelve patients with NP-C disease. To quantify their response, patients were examined by means of clinical rating scales: the Scale for the Assessment and Rating of Ataxia (SARA), the Spinocerebellar Ataxia Functional Index (SCAFI) and a modified Disability Rating Scale (mDRS). Scales were administered before the therapy with acetyl-DL-leucine, after one month of therapy and after one month of washout. Subjective improvement was assessed by the EuroQuality of Life questionnaire with visual analog scale (EQ-5D-5L). This first study found that with acetyl-DL-leucine therapy, clinical scales SARA and mDRS, as well as subtests of the SCAFI (i.e. 8-Meters walking time and 9-Hole pegboard test of the dominant and non-dominant hand) significantly improved. Moreover, family members described an increase of patients’ independent daily activities, as well as a diminishing of the intensity of square wave jerks. The conclusion of the first study was therefore that administration of acetyl-DL-leucine reduces cerebellar symptoms in patients with NP-C disease.

As postural imbalance is also a well-known feature of NP-C disease, the second aforementioned study evaluated whether an impairment of the vestibular system (canal and otolith function) contributes to the impairment of stance and gait in patients with NP-C disease. Patients were examined by means of the video-head impulse test to evaluate the angular
horizontal vestibulo-ocular reflex, cervical and ocular vestibular evoked myogenic potentials for otolith function and posturography.

The second study found that, contrary to other inherited metabolic disorders, such as Gaucher disease, vestibular function is intact in NP-C disease, implying that postural imbalance is of cerebellar origin, as was also shown by frequency analyses of posturographic data.

All in all, these two studies improve our knowledge of the treatment and pathophysiology of NP-C disease.
Introduction

1.1 Niemann-Pick type C disease

Niemann-Pick type C (NP-C, ORPHA no. 646) is a progressive, autosomal recessive, lysosomal storage disorder with a prevalence of 1:100,000 (Orphanet: Reports, 2015). Over 200 different mutations have been identified in NPC1 (95% of cases) or NPC2 (= 4% of cases) genes for proteins important in intracellular trafficking and transport of cholesterol and glycolipids (Alavi et al., 2013; Carstea et al., 1997; Fusco et al., 2012; Maue et al., 2012; Naureckiene et al., 2000; Park et al., 2003; Steinberg et al., 1994). Defects result in a cellular accumulation of endocytosed unesterified cholesterol, sphingomyelin (Butler et al., 1993), glycosphingolipids and sphingosine (te Vruchte et al., 2004) in the late endosome/lysosome, leading to functional disturbance of neural and non-neural tissues (Vanier and Millat, 2003). Based on the age of the first manifestation, the disease can be divided into early-infantile (< 2 years), late-infantile (2 to < 6 years), juvenile (6 to < 15 years), and adolescent/adult (≥ 15 years) forms (see Figure 1). A primary characteristic of NP-C disease is its phenotypic heterogeneity, comprising systemic, neurologic and psychiatric signs, that are not disease specific, arise at different ages, and progress at different rates (Garver et al., 2007; Iturriaga et al., 2006; Patterson et al., 2013; Vanier, 2010).

![Systemic involvement and Neurological involvement](image)

**Figure 1.** Schematic depiction of the clinical findings in Niemann-Pick type C disease. Adapted from (Vanier, 2015), with permission.
The diagnosis is established based on the filipin staining of cultured skin fibroblasts, showing pathological cholesterol storage and subsequently confirmed by molecular mutation analysis of the NPC1 and NPC2 genes (see Figure 2) (Patterson et al., 2012). Moreover, oxysterole plasma levels (Porter et al., 2010), targeted new-generation sequencing technologies (McKay et al., 2014) and plasma lysosphingomyelin levels (Welford et al., 2014) are promising biomarkers both for diagnosing and monitoring treatment response, respectively.

Figure 2. Accumulation of unesterified cholesterol visualized by filipin staining and fluorescence microscopy in skin fibroblasts cultured in presence of low-density lipoprotein. (a) Classic Niemann–Pick type C (NP-C) cell line; (b) variant NP-C cell line; (c) normal cell line. Adapted from (Vanier and Millat, 2003), with permission.

1.1.1 Neurological manifestations and their neuropathological, morphological and functional correlates

The most common neurologic finding in patients with NP-C disease is cerebellar ataxia (Patterson et al., 2013). Clinically, patients feature stance and gait ataxia with subsequent falls, clumsiness, dysmetria and dysdiadochokinesia. The ataxia in NP-C disease results from progressive Purkinje cell loss. Purkinje cells are the only neurones projecting out of the cerebellar cortex (Sarna et al., 2003). This is accompanied by microglial activation and monocyte recruitment to the brain.

Tremor is also a common finding, being mainly of cerebellar origin, but having also dystonic, myoclonic and choreiform components (Floyd et al., 2007). Remaining neurologic manifestations feature dystonia, dysarthria (caused by both cerebellar and cranial nerve
involvement), dysphagia, cognitive impairment, seizures, myoclonus and gelastic cataplexy (Patterson et al., 2013, 2015). These clinical symptoms indicate a progressive neurodegeneration.

On the cell level, ballooned neurons distended due to lipid storage, axonal spheroid formation, ectopic dendritogenesis, and demyelination can be seen (Bu et al., 2002; Sturley et al., 2004; Walkley and Suzuki, 2004). Moreover, neurofibrillary tangles (NFTs) (Suzuki et al., 1995), strongly reacting to antibody to tau-protein (Love et al., 1995), similar to those found in Alzheimer’s disease (AD) has been found. In NP-C, however, the tau-protein is predominantly distributed in subcortical structures, such as basal ganglia, thalami and hypothalami. Thus, both NP-C and AD can be classified as tauopathies (Williams, 2006), as in both, an increased CSF T-tau level can be found (Mattsson et al., 2011).

Neuroimaging studies also demonstrated a neurodegeneration in NP-C. Prior imaging studies showed grey matter reductions in subcortical and cortical areas, leading to general cerebral atrophy and thinning of the corpus callosum in NP-C disease (Walterfang et al., 2011; Walterfang et al., 2013). An increased signal in periatrial white matter, reflecting secondary demyelination, is also a common finding (Huang et al., 2011). Also, an atrophy of the cerebellar vermis has been described in severe cases of NP-C disease (Fusco et al., 2012).

The loss of the cerebellar grey matter tracks with disease severity and horizontal saccadic function, which is a measure of the functional integrity of brainstem and its efferents, inclusive cerebellar vermis (Bowman et al., 2015). Reduced saccadic gain has been previously correlated with the loss of cerebellar volume (Walterfang et al., 2013).

1.1.1.1 Cerebellar involvement, assessed by posturography
To maintain postural balance, the intact functions of the cerebellum, vestibular system and proprioception are needed (Brandt et al., 2012). In clinical practice, posturography can be administered to assess these balance components (Krafczyk et al., 2006). In addition, a precise topo-anatomical diagnosis can be created based on the evaluation of the presence and direction of the sway and visual stabilisation in conditions with eyes open/closed (Romberg quotient, RQ) (Diener et al., 1984) (see Table 1).
Table 1. Flow diagram for the topo-anatomical diagnosis of ataxia of stance. Adapted from (Diener et al., 1984), with permission. Abbreviations: RQ Romberg quotient AP antero-posterior LAT lateral

1.1.2 Ocular motor manifestations

Vertical supranuclear saccade palsy (VSSP) represents a cardinal symptom of NP-C disease at all disease stages, often being the initial sign of the disease. This finding was observed in 70% of patients from an international disease registry (Patterson et al., 2013) and in 81% of patients, found in a large-scale observational study (Garver et al., 2007). The saccadic dynamics in the course of NP-C disease has provided a measure of the therapeutic effect of miglustat, called horizontal saccadic eye movement (HSEM)-α, which is a slope of the linear regression line of peak duration vs. amplitude, identifying it as a treatment for this disorder (Patterson et al., 2007).
Introduction

Vertical eye movements are affected much earlier in the course of disease than horizontal ones (Abel et al., 2012; Rottach et al., 1997). This is due to the selective functional impairment of vertical burst neurons leading to neuronal loss in the rostral interstitial nucleus of medial longitudinal fascicle (riMLF). In contrast, horizontal saccades seem not to be impaired because premotor burst neurons in the paramedian pontine reticular formation (PPRF), the interstitial nucleus of Cajal (INC) and ocular motoneurons remain intact (for anatomical depiction, see Figure 3). This was demonstrated in a histopathological examination of the brain of a patient with NP-C disease (Solomon et al., 2005).

Figure 3. Depiction of the ocular motor nuclei in a sagittal section of the monkey brainstem. The paramedian pontine reticular formation (PPRF) (shaded region in pons), contains premotor excitatory burst neurons (EBN) for horizontal saccades (black oval in lower PPRF). The medullary reticular formation (Med RF), contains premotor inhibitory burst neurons (IBN) (black oval in upper Med RF). The asterisk just caudal to the cranial nerve VI (CN VI) rootlets depicts the location of the omnipause neurons in the raphe interpositus. Abbreviations: PC, posterior commissure; riMLF, rostral interstitial medial longitudinal fasciculus; INC, interstitial nucleus of Cajal; CN III, oculomotor nerve fascicle; III, oculomotor nucleus; IV, trochlear nucleus; MLF, medial longitudinal fasciculus; VI, abducens nucleus; CN VI, abducens nerve rootlets; NRTP, nucleus reticularis tegmenti pontis. Courtesy of Jean Buttner-Ennever, adapted from (Rucker et al., 2011), with permission.
Introduction

Upward, but especially downward saccades are slow, leading to an initial downward saccade palsy. Recent monkey and human studies identified a calretinin-positive excitatory input to motor centers mediating upgaze, arising from premotor centers: INC, riMLF and y-group (see Figure 4) (Che Ngwa et al., 2014; Zeeh et al., 2013, 2015). The lack of this input in downgaze pathways might explain the accentuated downgaze deficits in NP-C disease (Adamczyk et al., 2015). Calretinin is a calcium-binding protein, and in combination with parvalbumin and perineuronal nets can contribute to the future identification and subsequent analysis of the upgaze vs. downgaze ocular motor disturbances (Adamczyk et al., 2015).

![Figure 4. Calretinin input is restricted to motoneurons for upward eye movements in the monkey.](image)

Figure 4. Calretinin input is restricted to motoneurons for upward eye movements in the monkey. Summary diagram depicting the premotor pathways for upward eye movements (red), which are associated with calretinin. Abbreviations: RIMLF, rostral interstitial nucleus of the medial longitudinal fascicle; INC, interstitial nucleus of Cajal; CCN, central caudal nucleus; nIII, oculomotor nucleus; nIV, trochlear nucleus; LP, levator palpebrae; SO, superior oblique; MR, medial rectus; IR, inferior rectus and SR, superior rectus motoneurons. Adapted from (Zeeh et al., 2013), with permission.
The downward saccadic palsy has also been explained by a bilateral innervation of elevator muscles superior rectus and inferior oblique and unilateral (ipsilateral) innervation of the depressor muscles inferior rectus and superior oblique (Salsano et al., 2012). Thus, further studies are needed to clarify the specific patterns of saccadic degenerations in neurodegenerative disorders.

Up to now, ocular motor systems such as saccades or vestibulo-ocular reflex (VOR) have been investigated in a small number of patients (N<9) with NP-C disease (Abel et al., 2012; Rottach et al., 1997; Solomon et al., 2005), and as such systematic, exploratory, large-scale oculomotor studies in NP-C are still missing. Such studies will help finding reliable ocular motor parameters, which could be used as biomarkers in disease progress, and potentially be useful in monitoring treatment response.

1.1.3 Psychiatric manifestations
Psychiatric signs and symptoms often precede the neurologic manifestation (Maubert et al., 2013). The whole range of psychiatric symptoms include schizophrenic symptoms (paranoid delusions, auditory hallucinations, interpretative thoughts, disorganisation), depression, bipolar disorder, obsessive-compulsive behavior, behavioral disorders such as hyperactivity, agitation, aggressivity, mutilations, and sleep disorders. An underlying NP-C etiology may be suspected when patients do not respond to psychiatric treatment, or if there is aggravation or confusion under the neuroleptic therapy, together with observable catatonia, or seizures (Faludi et al., 2013; Maubert et al., 2013; Tyvaert et al., 2005).

Neurologic symptoms, appearing later on in the course of disease are often explained as an adverse event of the antipsychotic medication. The extreme heterogeneous, non-specific psychiatric manifestations lead to underdiagnosing of NP-C disease in psychiatric departments, leading to a considerable delay of 7 years on average between the initial symptoms and the establishing of the diagnose (Patterson et al., 2013).
1.1.4 Systemic signs

The systemic symptoms comprise enlargement of visceral organs, with neonatal jaundice and hepatomegaly in early-infantile forms and splenomegaly in juvenile and adult-forms. In older-onset cases, (hepato-)splenomegaly is usually asymptomatic, but probably present in almost 90% of all cases (Sévin et al., 2007). Pulmonary infiltration with foam cells is usually seen in patients with early-onset disease (Griese et al., 2010) or those with NPC2 mutations (Bjurulf et al., 2008). Systemic involvement, when present, usually precedes neurologic manifestations, suggesting an unknown difference in the pathophysiological mechanisms underlying visceral symptoms compared to those contributing to neurologic symptoms (Vanier, 2010).

1.1.5 Therapy with miglustat

The goal of the treatment of NP-C disease is a stabilisation or a reduction in the progression rate of neurologic manifestations. The iminosugar miglustat (Zavesca™) is an inhibitor of glucosylceramide synthase, that is an enzyme that catalyzes the first committed step in glycosphingolipid biosynthesis (Platt et al., 1994).

Miglustat slows down the progression rate of the neurologic manifestations in patients with NP-C disease. It is the only disease-specific drug approved for the therapy of NP-C disease (Patterson et al., 2007). Due to its small molecular weight, miglustat distributes easily after oral ingestion, and is able to pass the blood-brain barrier, which is supposed to be a key-factor in the treatment of neurologic symptoms. However, the effect of treatment starts with a delay of 6 to 12 months, and it may be even longer in patients with late-onset disease (Patterson et al., 2012). Data from miglustat-treated patients (n = 92) included in the international NP-C registry suggest that therapy with miglustat longitudinally stabilizes or improves neurologic manifestations (Fecarotta et al., 2015; Patterson et al., 2013). Stabilization of dysphagia is of particular importance because it is a high-risk factor for aspiration pneumonia (Walterfang et al., 2012).

A longitudinal imaging study comparing patients on miglustat therapy versus untreated patients showed that untreated patients with NP-C appeared to loose cerebellar grey and white matter, bilateral thalamic volume and right caudate volume faster than miglustat-treated patients (Bowman et al., 2015). These changes also correlated with changes in clinical rating scales.
Although miglustat demonstrates a good risk-safety profile (Brand et al., 2015; Hollak et al., 2009), gastrointestinal adverse events, such as diarrhea and flatulence are quite common. However, this tend to decrease in frequency and intensity over time (Champion et al., 2010).

1.2 Acetyl-DL-leucine

Acetyl-DL-leucine is an acetylated derivative of the essential amino-acid leucine. It has been used for the symptomatic treatment of vertigo and dizziness in France since 1957. Clinical experience has shown that it is a well-tolerated and safe drug without serious adverse effects (Lacour 1985; Léger et al. 1986; Neuzil et al. 2002; Pierre Fabre Médicaments, Castres, France). Acetyl-DL-leucine is able to cross the blood-brain barrier, explaining the reported effects and treatment responses in animal models and patients with unilateral vestibular loss (UVL) and cerebellar ataxia.

The effects of acetyl-DL-leucine on the activity of the medial vestibular nucleus (MVN) and vestibular-related networks have been measured electrophysiologically in an UVL guinea pig model (Vibert and Vidal, 2001a). The nature of response depended on the resting membrane potential. Acetyl-dl-leucine acted mainly on abnormally hyperpolarized and/or depolarized MVN neurons, by bringing back their membrane potential towards a mean value of −65 to −60 mV. Because of this stabilizing effect, acetyl-DL-leucine reduced the asymmetry within the vestibular-related networks caused by the UVL, decreasing the activity of the MVN neurons on the hyperactive intact side and increasing activity on the silent lesioned side, without affecting the neurons with a normal membrane potential. This mechanism is most likely mediated by its direct interactions with membrane phospholipids such as phosphatidylinositol-4,5-bisphosphate, which influences ion channel activity (Suh and Hille, 2008).

Studies in humans on the action of acetyl-DL-leucine were in accordance with the animal studies (Ferber-Viard et al., 2009). It has been shown that the effect depended on the presence of vestibular compensation (VC) before the labyrinthectomy. In patients with almost complete vestibular lesion prior to surgery, VC had taken place before the surgery, with vestibular neurons having regained a normal resting potential. In this group of UVL patients, no effect of the therapy was observed. This study has shown that acetyl-DL-leucine can ameliorate the static component of the VC following vestibular deafferentation, without having an effect on the dynamic component.
Furthermore, a prior $^{18}$F-Fluoro-desoxyglucose ($^{18}$F-FDG)-µPET study in an UVL rat model investigating the regional cerebral metabolic rate for glucose (rCGMglc) revealed that only L-isomer or DL-racemate, but not N-acetyl-D-leucine caused a significant acceleration of VC, acting in a dose-dependent manner. Moreover, only L-isomer caused a significant increase of rCGMglc in the vestibulocerebellum and a decrease in the posterolateral thalamus and subthalamic region (see Figure 5) (Günther et al., 2015). This is supported by a recent study, focused on the examining of the pharmacological effects of acetyl-DL-leucine in an UVL cat model, showing that L-isomer is an active component of the DL-racemate, since it significantly accelerates the vestibular compensation process (Tighilet et al., 2015).

Studies showed that the calcium homeostasis is dysregulated in NPC disease, similar to spinocerebellar ataxias (SCAs), as reflected in the calcium depletion in the late endosome/lysosome (Lloyd-Evans et al., 2008). This leads to the functional disturbance of Purkinje cells. Since the input from Purkinje cells and mossy/climbing fiber collaterals controls the action potential of the vestibular and the cerebellar nuclei (Witter et al., 2011), which in turn project to the brainstem, thalamus and spinal cord (Highstein and Holstein, 2006), acetyl-DL-leucine may act through afferent and efferent projections on upstream and downstream structures, thus influencing movement control.
Introduction

Figure 5. Comparison of the N-acetyl-L-leucine effect on regional cerebral glucose metabolism following unilateral labyrinthectomy and sham unilateral labyrinthectomy.

Regional cerebral glucose metabolism (rCGM) on days 1, 3, 7 and 15 between groups treated with N-acetyl-L-leucine (24 mg i.v. per rat) after unilateral labyrinthectomy (UL) and after sham UL (i.e., without inner ear damage). A) rCGM was significantly decreased in the posterolateral thalamus bilaterally on days 3 and 7 and the contralesional subthalamic region on day 3 in the UL-group as compared to the sham UL-group. B) On days 3 and 7 rCGM was significantly increased in the vestibulocerebellum bilaterally in the UL-group. Abbreviations: R, right; L, left; C, caudal; Ro, rostral; Ce, cerebellum; St, subthalamic region; Th, thalamus. P-value < 0.001. Adapted from (Günther et al., 2015), open access.
A clinical study in patients with degenerative cerebellar ataxia of different etiologies has shown a positive effect on the cerebellar symptomatology, without causing any side effects (Strupp et al., 2013). Patients improved significantly on 6 out of 8 sub-scores of the Scale for Assessment and Rating of Ataxia (SARA) (gait, speech, finger-chase, nose-finger-test, rapid-alternating-movements, and heel-to-shin). The evaluation of the Spinocerebellar Ataxia Functional Index (SCAFI) showed a better performance in 3 out of 4 elements (8-m-walking-time, 9-Hole-Peg-Test of the dominant hand and PATA rate). Of note, the objective improvement was additionally reflected in the increased quality of life during treatment.

Another clinical study demonstrated an improved coefficient of variation of stride time in the gait analysis in 14 out of 18 patients with cerebellar ataxia (Schniepp et al., submitted). The improvement of variability was restricted to the condition of slow walking, where walking stability is thought to critically rely on the sensory integration function of the cerebellum (Schniepp et al., 2012).

In contrast, in a case-series with 10 patients with degenerative cerebellar ataxia, no improvement in SARA was observed (Pelz et al., 2015). However, 7 out of 10 patients described a subjective improvement on medication. Since at the time of this study, the acetyl-DL-leucine tablets were not available, a liquid formulation of 5 g once a day was administered, which may account for the failure to confirm the therapeutic benefit.

All in all, the beneficial effect of therapy with acetyl-DL-leucine in patients with NP-C needs to be confirmed by a randomized, long-term, placebo-controlled, double blind, 2 way cross-over phase II clinical trial.
Aims of this thesis

There is no symptomatic or disease-specific therapy for cerebellar ataxia. So far, symptomatic treatments with potassium channel blockers azetazolamide and aminopyridines have been investigated, improving some of the ataxia symptoms (Claassen et al., 2013a; Kalla et al., 2007; Schniepp et al., 2011; Strupp et al., 2003, 2004, 2011). The only recommended therapy in ataxias is intensive physiotherapeutic training (Ilg et al., 2014). As such, new therapeutic options are a high priority.

This cumulative thesis consists of two manuscripts, of which the first one has been published in the peer-reviewed journal Neurology®. The second one has also been submitted for publication in a peer-reviewed journal and is under review. The aims of this cumulative thesis are: First, to explore the effects of acetyl-DL-leucine on cerebellar ataxia symptoms, eye movements, and quality of life in patients with NP-C disease. This agent has been shown to improve cerebellar symptoms in patients with cerebellar ataxia of different etiologies (Strupp et al., 2013). Second, to address the question of whether there is a vestibular deficit in NP-C disease or not, which could contribute to the well-known postural imbalance in this disorder.
Acetyl-DL-leucine in Niemann-Pick type C: a case series

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AUTHOR CONTRIBUTIONS

T. Bremova: design of the study, conducting experiments, data analysis, data interpretation, drafting/revising the manuscript. V. Malinová: patient recruitment/data interpretation/revising the manuscript for important intellectual content. Y. Amraoui: patient recruitment/revising the manuscript for important intellectual content. E. Mengel: revising the manuscript for important intellectual content. J. Reinke: revising the manuscript for important intellectual content. M. Kolníková: patient recruitment/revising the manuscript for important intellectual content. M. Strupp: study concept/revising the manuscript for important intellectual content.

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ABSTRACT

Objective: To assess the effects of the modified amino-acid acetyl-DL-leucine (AL) on cerebellar ataxia, eye movements, and quality of life of patients with Niemann-Pick type C (NP-C) disease.

Methods: Twelve patients with NP-C disease were treated with AL 3 g/day for 1 week and then with 5g/day for 3 weeks with a subsequent wash-out period of 1 month. The Scale for the Assessment and Rating of Ataxia (SARA), the Spinocerebellar Ataxia Functional Index (SCAFI), the modified Disability Rating Scale (mDRS), EuroQol 5Q-5D-5L, and the visual analog scale (VAS) were administered. Measurements took place at baseline, after 1 month of therapy, and after 1 month of wash-out.

Results: The SARA score changed from the baseline (median [±SD, interquartile range]) of 10.8 (11.2, 8-24.6) to 7.0 (10.7, 5.6-19.6) on medication (difference: 3.8 points) and 10.5 (11.5, 7.1-23.9) after washout (difference: 3.5 points) ($p = 0.000412$; post hoc $p = 0.003$ between baseline and on-medication, and on-medication and wash-out $p = 0.005$). The SCAFI subscore 9-Hole Peg Test (9HPT) for dominant hand, mDRS score, and VAS score also improved on medication. No side effects except transient dizziness in one patient were reported.

Conclusions: Treatment with AL improved ataxic symptoms in patients with NP-C without relevant side effects, thus showing a reasonable risk-benefit profile.

Classification of evidence: This study provides Class IV evidence that AL improves cerebellar symptoms and quality of life in patients with NP-C disease.
Acetyl-DL-leucine in NP-C

INTRODUCTION

Niemann-Pick type C (NP-C) is a hereditary lysosomal storage disease characterized by progressive neurologic deterioration and premature death (Vanier, 2010). The disease presents with systemic, psychiatric and neurologic symptoms, including cerebellar ataxia, most pronounced in juvenile and adult patients (Mengel et al., 2013; Patterson et al., 2012, 2013).

The current disease-specific therapy approved for NP-C is miglustat (Zavesca; Actelion Pharmaceuticals Ltd, Allschwil, Switzerland), which targets sphingolipid synthesis and storage and thus slows the progression of the disease (Vanier, 1999). However, because of the progressive and irreversible nature of the disease, additional symptomatic treatment is needed to improve functioning and quality of life and to alleviate the burden of disease.

The prior study assessing the effect of therapy with the acetylated derivative of a natural amino-acid, acetyl-DL-leucine (AL) (Tanganil; Pierre Fabre, Castres, France) in patients with cerebellar ataxia of different etiologies suggested the beneficial effect of this agent (Strupp et al., 2013). In this study, we evaluated the effect of therapy with AL in patients with genetically and/or biochemically proven NP-C disease.
SUBJECTS AND METHODS

Level of evidence. The aim of this Class IV evidence study was to evaluate the effect of AL 3 g/day for 1 week (Tanganil 500 mg) and then 5g/day for the following 3 weeks (in total, 1 month) on cerebellar function, ocular motor function, and subjective satisfaction.

Standard protocol approvals, registration, and patient consents. This case series was an observational study. All patients and/or guardians of patients gave their informed consent for the compassionate use of AL. Signed patient consent-to-disclose forms were also obtained for videos of patients and their family members.

Patients. Ten patients with genetically confirmed and 2 patients with biochemically confirmed NP-C disease (5 females, mean age [±SD] 22.9 ± 4.9 years, mean disease duration 13.7 ± 4.1 years, mean age when diagnose was established 17.7 ± 5.3 years) were included. The clinical characteristics of the patients are given in table 1.

Table 1. Baseline characteristics of the subjects included in the study.

<table>
<thead>
<tr>
<th>Patient no./ Sex/Age</th>
<th>Age of onset/ diagnosis</th>
<th>Medication</th>
<th>Genotype</th>
<th>Neurological and psychiatric findings</th>
<th>Internal manifestation/other findings</th>
<th>Ocular motor findings</th>
<th>MRI findings</th>
<th>MoC</th>
<th>IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/13</td>
<td>4.5/10</td>
<td>Levetiracetam 1750 mg/d, valproate 750 mg/d, miglustat 400 mg/d</td>
<td>NPC1: c.3182T&gt;C, c.3557G&gt;A</td>
<td>a, b, c, d, e, f, g, j, k, o, p, s, t</td>
<td>Medium grade splenomegaly, PEG</td>
<td>a, b, c, d, e</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/F/23</td>
<td>3/19</td>
<td>Levetiracetam 300 mg/d, sertraline 50 mg /d, donepezil 10 mg/d, clonazepam</td>
<td>NPC1: c.2861C&gt;T, c.3557G&gt;A</td>
<td>a, b, c, d, e, f, h, i, j, k, n, o, s, t</td>
<td>Mild splenomegaly, cachectic habitus</td>
<td>a, c, d, a, b</td>
<td>NcP</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Age</td>
<td>Gender</td>
<td>Symptoms and Treatments</td>
<td>NPC1 Mutations</td>
<td>Initial Manifestation</td>
<td>NeP</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3/F/26</td>
<td>0.5 mg/d, miglustat 600 mg/d</td>
<td>3/F</td>
<td>Valproate 600 mg/d, miglustat 500 mg/d</td>
<td>NPC1: c.1935dupT, c.2861C&gt;T</td>
<td>PEG, tracheostomy, cachectic habitus</td>
<td>NeP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/F/32</td>
<td>74</td>
<td>4/F</td>
<td>Sertraline 50 mg/d, vitamin B1, B6, B12 1x/week</td>
<td>NPC1: c.1028G&gt;A, c.2198C&gt;G</td>
<td>Initial manifestation after birth of her child</td>
<td>NeP</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5/F/25</td>
<td>60</td>
<td>5/F</td>
<td>Miglustat 600 mg/d</td>
<td>NPC1: c.3019C&gt;G, c.3592-7_3754-3delCTT TT</td>
<td>Mild splenomegaly</td>
<td>NeP</td>
<td></td>
<td></td>
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<tr>
<td>6/M/24</td>
<td>60</td>
<td>6/M</td>
<td>Miglustat 600 mg/d, hearing devices bilaterally</td>
<td>NPC1: c.2474A&gt;G, c.3160G&gt;A</td>
<td>Splenomegaly</td>
<td>NeP</td>
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<td>7/M/20</td>
<td>46</td>
<td>7/M</td>
<td>Risperidon 0.5 mg/d, miglustat 600 mg/d</td>
<td>mutation in NPC1 and NPC2 genes found</td>
<td>Mild hepatosplenomegaly</td>
<td>NeP</td>
<td></td>
<td></td>
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<tr>
<td>8/M/26</td>
<td>40</td>
<td>8/M</td>
<td>Levetiracetam 200 mg</td>
<td>NPC1: c.1232G&gt;A</td>
<td>Hepatosplenic</td>
<td>NeP</td>
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### Acetyl-DL-leucine in NP-C

<table>
<thead>
<tr>
<th>Date</th>
<th>Date</th>
<th>Drug(s)</th>
<th>Dose</th>
<th>Mutation(s)</th>
<th>Diagnosis(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/M/20</td>
<td>5/16</td>
<td>Ramipril, metronidazole, miglustat</td>
<td>0.125 mg/d, 600 mg/d</td>
<td>c.2861C&gt;T, c.3557G&gt;A</td>
<td>Splenomegaly, Hepatosplenomegaly, Microcytic anemia, Arterial hypertension, Spleno-Megaly, Mild splenomegaly</td>
</tr>
<tr>
<td>10/M/2</td>
<td>12/17</td>
<td>Lamotrigine, Piracetam, Gingko biloba, Miglustat</td>
<td>350 mg/d, 3600 mg/d, 600 mg/d</td>
<td>c.808delG, c.2861C&gt;T</td>
<td>Splenomegaly, Hepatosplenomegaly, Microcytic anemia, Arterial hypertension, Spleno-Megaly, Mild splenomegaly</td>
</tr>
<tr>
<td>11/M/2</td>
<td>14/15</td>
<td>Miglustat, Ginko biloba, Vitamin E, Piracetam</td>
<td>400 mg/d, 3600 mg/d</td>
<td>c.1723delG, c.2861C&gt;T</td>
<td>Splenomegaly, Hepatosplenomegaly, Microcytic anemia, Arterial hypertension, Spleno-Megaly, Mild splenomegaly</td>
</tr>
<tr>
<td>12/F/1</td>
<td>10/11</td>
<td>Vitamin D, Miglustat</td>
<td>500 I.E./d, 600 mg/d</td>
<td>No mutation in NPC1, No mutation in NPC2</td>
<td>Splenomegaly, Hepatosplenomegaly, Microcytic anemia, Arterial hypertension, Spleno-Megaly, Mild splenomegaly</td>
</tr>
</tbody>
</table>

**Abbreviations:** CT = Computer Tomography, MRI = Magnetic Resonance Imaging, MoCA = Montreal Cognitive Assessment, UEx = upper extremities, LEx = lower extremities, PEG =
Acetyl-DL-leucine in NP-C

percutaneous endoscopic gastrostomy, NP = not performed, NcP = not capable of performing the
task due to physical limitations

\(^a\)Neurological and psychiatric findings: a = epilepsy; b = ataxic stance and gait; c =
dysmetria/tremor UEx; d = dystonia; e = contractures of Achilles tendons; f = dysphagia; g =
gelastic cataplexy; h = dyskinesias; i = emotional instability; j = clonus LEx; k = hyperreflexia; l
= stuttering; m = hearing impairment; n = confined to a wheelchair; o = excessive salivation; p =
hypomimia; r = organic psychosis; s = dysarthria; t = cognitive impairment; u = logorrhea; v =
complete anarthria

\(^b\)The total possible MoCA score is 30 points; a score of 26 or above is considered normal. Data
at baseline.

\(^c\)Ocular motor findings: a = slow vertical saccades; b = vertical saccade paresis downward; c =
impaired vertical smooth pursuit; d = fixation instability, e.g. square wave jerks; e = impaired
vertical optokinetic nystagmus; f = strabism

\(^d\)MRI findings: a = supratentorial enlarged liquor spaces; b = generalized atrophy; c = cerebellar
atrophy; d = brainstem atrophy; e = leukodystrophy; n = normal findings

\(^e\)Positive Filipin Staining (variant type)

\(^f\)Family relatives (mothers are cousins)

\(^g\)Full Scale IQ as tested by Wechsler Adult Intelligence Scale Revised or Wechsler Intelligence
Scale for Children-IV

**Evaluations.** To evaluate the overall neurologic status in NP-C disease, the modified Disability
Rating Scale (mDRS) by Pineda et al., 2010 was applied; the mDRS is a 4-domain scale
(ambulation, manipulation, language and swallowing) in an extended form (Iturriaga et al.,
2006), which also includes seizures and ocular movements, that assesses the severity of the
disease and monitors the effect of treatment. The following cerebellar function evaluations were
administered: (1) the Scale for the Assessment and Rating of Ataxia (SARA) (Schmitz-Hübsch
et al., 2006; Subramony, 2007), an eight-item clinical rating scale (gait, stance, sitting, speech, fine
motor function and taxis; range 0–40, where 0 is the best neurological status and 40 the worst);
and (2) the Spinocerebellar Ataxia Functional Index (SCAFI), comprising the 8-m- walking time
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(8MW) performed by having patients walk twice, as quickly as possible, from one line to another excluding turning, the 9-Hole Peg Test (9HPT) and the number of “PATA” repetitions over 10 seconds (PATA) (Schmitz-Hübsch et al., 2008).

Subjective impairment and quality of life were evaluated by using the EuroQol 5Q-5D-5L questionnaire (EQ-5D-5L) (Devlin and Krabbe, 2013) and the visual analog scale (VAS). To assess the effect of the therapy on ocular motor function, 3-dimensional video-oculography (EyeSeeCam) (Glasauer et al., 2003) was used to measure the peak velocity of saccades, gain of smooth pursuit, slow phase velocity of gaze-evoked nystagmus (gaze-holding function) (SPV), SPV of optokinetic nystagmus, and gain of horizontal vestibulo-ocular reflex at each visit (Schneider et al., 2009). To evaluate the potential treatment effect, administration of the SCAFI scale in patients with NP-C disease was recorded on video (for 9HPT examination see videos 1 and 2 on the Neurology® Web site at Neurology.org). Measurements and questionnaire administration took place at baseline, after 1 month of therapy with AL (on day 30 ± 1 day), and after a 1-month washout period (on day 60 ± 2 days). To evaluate the cognitive state, the Wechsler Adult Intelligence Scale-Revised (WAIS-R) or Wechsler Intelligence Scale for Children-IV (WISC-IV) (Grove, 1950), and Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005), assessing different cognitive domains, including attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation with a maximum of 30 points and a cut-off score of 26, were administered once at baseline ± 1 month (see table 1).

Patients and their parents were asked about their subjective improvement on medication; videos of their subjective evaluation of the effect treatment and possible side effects (see video 3) were also recorded. Drug administration and neurological examination (with one exception: WAIS-R/WISC-IV) were performed by one examiner (T.B.).

Statistical analysis. Statistical analysis and figure design were performed using SPSS version 22.0.0 (IBM, Armonk, NY). Differences were considered significant if \( p < 0.05 \). Because data were not normally distributed, related-samples Friedman test with \( \chi^2 \) test statistics was run to determine whether there were differences in measured scores between baseline, on-medication and washout time-points. Post hoc analysis with the Wilcoxon signed-rank test was conducted with a Bonferroni correction. Spearman rank correlation coefficient was used to assess the relationships between tested variables. Patients who were not physically capable of performing
the particular score tasks were not included in the analysis.
RESULTS

Effects of AL on neurologic status. The total SARA score was 10.8 (11.2, 8-24.6) at baseline (median [±SD, IQR]), 7.0 (10.7, 5.6-19.6) after one month of medication (difference: 3.8 points), and 10.5 (11.5, 7.1-23.9) after one-month of wash-out (difference: 3.5 points) (for individual value changes, see table e-2, figures 1, A and B), indicating an improvement of cerebellar signs on medication ($\chi^2(2) = 15.591$, $p = 0.000412$). The post hoc testing revealed a statistically significant difference between the baseline and the on-medication scores ($p = 0.003$) and between the on-medication and the wash-out scores ($p = 0.005$), but no significant difference between the baseline and the wash-out scores ($p = 0.561$).
Figure 1. Effect of treatment with acetyl-DL-leucine 5 g/d on neurologic status in patients with Niemann-Pick type C. Individual and value changes on the Scale for the Assessment and Rating of Ataxia (SARA) (A, B) and the modified Disability Rating Scale (mDRS) (C, D). Measurements performed at baseline, after 1 month on medication with acetyl-DL-leucine 5 g/d, and after 1 month of wash-out. The total SARA score changed significantly from the baseline (median [±SD, IQR]) of 10.8 (11.2, 8-24.6) to 7.0 (10.7, 5.6-19.6) after one month of medication (difference 3.8 points) and 10.5 (11.5, 7.1-23.9) after one month of wash-out (difference 3.5 points), indicating an improvement of cerebellar signs on medication ($\chi^2(2) = 15.591, p = 0.000412$). The total mDRS score (median, [±SD, IQR]) was 10.0 (5.35, 7-23) at baseline, 9.0 (5.3, 6-23) on medication (difference 1 point), and 10.0 (5.4, 6-23) after 1 month of wash-out (difference 1 point). This change was statistically significant ($\chi^2(2) = 13.04, p = 0.001$). The length of the boxes (B, D) indicates the interquartile space (P25–P75), the horizontal line into the box represents the median (P50) and the whiskers indicate the adjacent values. The circle indicates the outlier. Figure modified for the purposes of this thesis.

The SCAFI 9HPT of the dominant hand changed significantly ($\chi^2(2) = 6.889, p = 0.032$), yielding significant differences between baseline and on medication ($p = 0.038$) as well as on medication and wash-out ($p = 0.033$), but not between baseline and wash-out ($p = 0.594$). There was a general trend for improvement of the 9HPT of the non-dominant hand ($p = 0.121$) and 8MW ($p = 0.178$) on medication. The PATA score did not change significantly between measurements ($p = 0.406$) (table e-2, figures 2, A-D).
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Figure 2. Effect of treatment with acetyl-DL-leucine 5 g/d on cerebellar status in patients with Niemann-Pick type C, as assessed by the SCAFI. Boxplot representation of cerebellar function, as assessed by the Spinocerebellar Ataxia Functional Index (SCAFI) subscore items 8-m Walk (8MW) test time (A), 9-Hole Peg Test time for dominant (9HPTD) (B) and non-dominant (9HPTN) (C) hand, and PATA word count in 10 seconds (D) before, during, and after therapy with acetyl-DL-leucine 5 g/d. The SCAFI 9HPTD item changed significantly ($\chi^2(2) = 6.889, p = 0.032$) yielding significant differences between baseline and on medication ($p = 0.038$) as well as on medication and wash-out ($p = 0.033$), but not between baseline and wash-out ($p = 0.594$). There was a general trend for improvement of 9HPTN ($p = 0.121$) and 8MW test ($p = 0.178$) on medication. The PATA score did not change significantly between measurements ($p = 0.406$). The length of the boxes indicates the interquartile space (P25–P75), the horizontal line into the box represents the median (P50) and the whiskers indicate the adjacent values. The circles indicate the outliers and the stars represent an extreme value. Figure modified for the purposes of this thesis.

The total mDRS score (median [±SD, IQR]) was 10.0 (5.35, 7-23) at baseline, 9.0 (5.3, 6-23) on medication (difference: 1 point), and 10.0 (5.4, 6-23) after one-month of wash-out.
Acetyl-DL-leucine in NP-C

(difference: 1 point) (table e-2 and figures 1, C and D). This change was statistically significant ($\chi^2(2) = 13.04, p = 0.001$). The post hoc testing revealed a statistically significant difference between the baseline and the on-medication scores ($p = 0.01$) and between the on-medication and the wash-out scores ($p = 0.024$), but no significant difference between the baseline and the wash-out scores ($p = 0.083$). There was a negative correlation between the neurologic and cognitive status when the baseline mean total SARA score and IQ score was analyzed ($\rho = -0.756, p < 0.05$). Correlation analysis also showed a trend for a significant association between MoCA and SARA scores ($\rho = -0.622, p = 0.055$) and MoCA and mDRS scores ($\rho = -0.579, p = 0.079$).

**Ocular motor function.** The mean peak velocity (±SD, range) of the vertical saccades was 55.0 °/s (67.2, 35.8-111.3) at baseline, 71.0 °/s (24.3, 44.0-82.0) on medication and 44.0 °/s (19.7, 33.0-50.0) after one month of wash-out, $p = 0.244$). The other ocular motor parameters tested also did not significantly change (overall comparisons): $p$ for peak velocity of horizontal saccades: 0.846, smooth pursuit gain: vertical: 0.554, horizontal: 0.115; $p$ for slow phase velocity (SPV) of gaze-evoked nystagmus: vertical: 0.761, horizontal: 0.717; $p$ for horizontal vestibulo-ocular reflex gain: 0.692; SPV of optokinetic nystagmus: vertical: 0.311, horizontal: 0.692.

**Quality of life.** Quality of life, as assessed by EQ-5D-5L, changed from a baseline of 0.62 (0.35-0.83) to 0.72 (0.43-0.83) on medication and 0.52 (0.19-0.85) without medication ($p = 0.459$) (see table e-2 and figure 3A). VAS changed significantly on medication ($p = 0.05$), rising from 30 (25-70) at baseline to 45 (35-80) on medication ($p = 0.02$). After one month of therapy, VAS decreased to 30 (20-60) ($p = 0.776$) (see table e-2 and figure 3B).

**Subjective evaluation.** Family and caregivers of 8 out of the 12 patients believed that there was improvement in one or more areas. Parents of 3 out of 12 patients described remarkable behavioral improvement in affect stabilization, cooperation, and ability to act independently in daily life (e.g. dressing, grooming, drawing). In 3 patients, subjective improvement of dysphagia was also reported (fewer swallowing problems whilst drinking and eating). In one patient with square wave jerks (SWJ), subjective improvement of fixation, as reported by the parents, was observed (see table e-2).
Figure 3. Effect of treatment with acetyl-DL-leucine 5 g/d on the quality of life of patients with Niemann-Pick type C. Boxplot representation of the value changes on the EuroQol 5Q-5D-5L questionnaire (EQ-5D-5L) (A) and the visual analog scale (VAS) (B). EQ-5D-5L, assessing the quality of life, changed from a baseline of 0.62 (0.35-0.83) to 0.72 (0.43-0.83) on medication and 0.52 (0.19-0.85) without medication ($p = 0.459$). VAS changed significantly on medication ($p = 0.05$), rising from a baseline of 0.30 (0.25-0.70) to 0.45 (0.35-0.80) on the treatment ($p = 0.02$). After one month of therapy, VAS decreased to 0.30 (0.20-0.60) ($p = 0.776$). The length of the boxes indicates the interquartile space (P25–P75), the horizontal line into the box represents the median (P50) and the whiskers indicate the adjacent values. Figure modified for the purposes of this thesis.

**Side effects.** One patient reported intermittent dizziness on the dosage of 5 g/day, which ceased after the dose was reduced to 3 g/day for one week. When the dosage was increased again, the symptoms did not recur.
DISCUSSION

The major findings of this case series are as follows: first, the modified amino-acid acetyl-DL-leucine (AL) had a significant effect on cerebellar signs and symptoms in patients with genetically and/or biochemically proven NP-C disease. Second, the improvement of neurologic status also led to a significant improvement of the quality of life of the patients and their family members. Third, the low frequency (1 out of 12) and the temporary nature of the adverse effects suggest a reasonable risk-benefit profile.

Acetyl-DL-leucine has been used in France since 1957 to treat acute vertiginous symptoms; however, despite a number of proposed hypotheses, including a stabilization of membrane potential, its pharmacologic and electrophysiologic modes of action have not yet been clarified (Ferber-Viard et al., 2009; Vibert and Vidal, 2001a; de Waele et al., 1990). A fluorodeoxyglucose (FDG)-µPET study in a rat model of acute unilateral vestibular lesions demonstrated a significant effect of the N-acetyl-L-leucine enantiomer on postural compensation by means of an activation of the vestibulocerebellum and a deactivation of the posterolateral thalamus (Günther et al., 2015). Clinically, the improvement of cerebellar symptoms in humans in a case series with cerebellar patients of different etiologies indicated the therapeutic efficacy of AL (Strupp et al., 2013). Furthermore, a PET study in patients with ataxia of different etiologies given AL demonstrated an increased metabolism in the midbrain and lower brainstem in responders (Becker-Bense et al., personal communication). Targeting vestibular together with cerebellar regions is probably one of the key actions of AL. Impaired central vestibular function in patients with NP-C is very likely in light of the well-known ocular motor (Abel et al., 2009) and hearing dysfunction (King et al., 2014), even though the vestibulo-ocular reflex, representing the peripheral vestibular function seems to be intact (Solomon et al., 2005). However, no evidence regarding vestibular function in patients with NP-C disease has been gained so far and its improvement might also be responsible for the positive effect of the therapy.

In our cohort of patients with NP-C, AL stabilized stance and gait with a lowered risk of falls, and improved dysmetria and intentional tremor, thus improving fine motor function. This had an impact on the daily activities of patients with NP-C, also reflected in increased quality-of-life scores on medication. Improvement of dysarthria and dysphagia was not a consistent finding, probably because of concomitant bulbar syndrome, and considerable impaired cognition, because this leads to absence of communication in some patients. This was also reflected in the fact that neurologically more severely affected patients also had a notably lower IQ with a lowered MoCA
score. Because patients with NP-C present with a heterogeneous symptomatology, ranging from palliative cases unable to act independently to a very mild presentation with isolated slow vertical saccades (Garver et al., 2007), the test results and reactions to treatment vary considerably. Cognitive impairment and psychiatric comorbidities, such as affective lability with pathologic crying or psychotic presentation with aggressive traits, and the abovementioned frequent generalized dystonia, and – in later stages – spasticity impeded clinical evaluation of the therapy effect. Of note, a slight stabilization of the affect, increase of drive, social interaction, and improved performance of complex tasks such as dressing or drawing, on medication was reported by parents and rehabilitation staff (see video 3). This might be a secondary effect of the positive influence on overall neurologic function; nevertheless, a specific, but as yet unclear effect on distinct areas responsible for higher cognitive functions and emotions, such as the frontotemporal lobe or the limbic system should also be taken into consideration.

No remarkable effect of AL therapy on ocular motor function has been noted, especially not on the supranuclear ocular motor centers in the brainstem; however, an improvement of fixation by diminishing the intensity of square wave jerks might suggest a positive effect on cerebellar ocular motor centers. This is in line with the previously shown and abovementioned increase of regional cerebral metabolic rate for glucose in the vestibulocerebellum (Günther et al., 2015) and brainstem (Becker-Bense et al., personal communication).

The only side effect of the medication was transient, dose-dependent dizziness in one of the 12 patients, thus demonstrating a good risk-benefit profile of the medication.

Our study has several limitations. First, this is an observational study with all its limitations and not a randomized, placebo-controlled trial. Therefore, a placebo effect or a training effect on components of ataxia assessment (e.g., 9HPT) cannot be ruled out. Second, the long-term efficacy of AL was not evaluated. Therefore, a longer-term, placebo-controlled, double-blind, randomized clinical trial of AL in a larger cohort of patients with NP-C is necessary to assess its safety and effects on disease progression. Third, in 2 of the 12 patients, no mutation was found.

This observational study demonstrated that AL had a positive effect on ataxia in patients with NP-C, improved their quality of life, and was well tolerated. Since the mechanism of the AL action is not thought to be NP-C specific, if AL showed benefit in a placebo-controlled trial in NP-C or any other disease with prominent ataxia, it might be generally useful across all ataxias.
REFERENCES

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DISCLOSURES
Dr. Bremova has received speaker’s honoraria from Actelion. Dr. Amraoui and Dr. Kolníková report no disclosures. Dr. Malinová received speaker’s honoraria from Actelion, Sanofi-Genzyme, Shire and Synageva. Dr. Mengel received speaker’s honoraria and consultant fees from Actelion, Genzyme, BioMarin, Shire HGT and Synageva. Dr. Reinke received speaker’s honoraria from BioMarin, Shire, Genzyme and Actelion. Dr. Strupp is Joint Editor-in-Chief of the Journal of Neurology, Editor-in-Chief of Frontiers of Neuro-otology and Section Editor of F1000. He received speaker’s honoraria from Abbott, UCB, GSK, TEVA, Biogen Idec, Pierre-Fabre, Eisai and HennigPharma.
### Supplemental material

**Supplemental Table e-2**

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Clinical assessment of patients by the modified Disability Rating Scale (mDRS), the Scale for the Assessment and Rating of Ataxia (SARA), the Spinocerebellar Ataxia Functional Index (SCAFI), comprising the 8-m-Walking-Time (8MW), 9-Hole-Peg-Test (9HPT) and the number of “PATA” repetitions over 10 s (PATA), the EuroQol 5Q-5D-5L questionnaire (EQ-5D-5L), and the visual analog scale (VAS) at baseline, on medication, and after one month of wash-out.

Abbreviations: mDRS modified Disability Rating Scale, SARA Scale for the Assessment and Rating of Ataxia, SCAFI Spinocerebellar Ataxia Functional Index, 8MW 8-m-Walking-Time, 9HPTD 9-Hole-Peg-Test of the dominant hand, 9HPTN 9-Hole-Peg-Test of the non-dominant hand, PATA PATA word repetition test in 10 s, EQ-5D-5L Euro Quality of Life Scale, VAS Visual Analog Scale

NcP not capable of performing the task due to physical limitations

NP not performed for other reasons

* Subjective improvement on medication, as assessed by parents: a = stance; b = gait; c = tremor; d = fine motor function; e = speech disturbance; f = swallowing; g = ocular movements, e. g. square wave jerks; h = social interaction; i = self-care, e. g. eating, grooming, dressing, going to the toilet
**Supplemental video legends**
Videos can be found on the attached CD at the end of this thesis.

Video 1
9-Hole-Peg Test of the dominant hand of patient 11 at baseline, on medication and after one month of wash-out.

Video 2
9-Hole Peg Test of the dominant hand of the patient 2 at baseline, on medication and after one month of wash-out.

Video 3
Interview with the parents of Niemann-Pick type C patients describing the effects of the treatment with acetyl-DL-leucine on the neurological function and acting in daily life.
Vestibular function in patients with Niemann-Pick type C disease

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² Department of Neurology, University Hospital Munich, Grosshadern Campus, Munich, Germany
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AUTHOR CONTRIBUTIONS

T. Bremova: idea for the study, design, data collection, data interpretation, statistical analysis, writing the manuscript. S. Krafczyk: data interpretation, revising the manuscript for important intellectual content. S. Bardins: figure design, revising the manuscript for important intellectual content. J. Reinke: revising the manuscript for important intellectual content. M. Strupp: revising the manuscript for important intellectual content.

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22.10.2016 The manuscript has been accepted for publication on 29th of July 2016, thereby after the submission of this dissertation under "Vestibular function in patients with Niemann-Pick type C disease article, DOI :10.1007/s00415-016-8247-4, 2016, Epub ahead of print, Springer Journal of Neurology, by Bremova T, Krafczyk S, Bardins S, Reinke J, Strupp M. "With permission of Springer".
Abstract
We investigated whether vestibular dysfunction is the cause or may cause or contribute to postural imbalance and falls in patients with Niemann-Pick type C disease (NP-C). Eight patients with NP-C disease and 20 healthy controls were examined using the video-based head impulse test (vHIT) and caloric irrigation to investigate horizontal canal function as well as ocular- and cervical vestibular evoked myogenic potentials (o- and cVEMP) and binocular subjective visual vertical estimation (SVV) for otolith function, and static posturography. There were no significant differences in vestibulo-ocular gain, caloric excitability, o-/cVEMP measures or SVV between the two groups. Posturographic total sway path (tSP) and root mean square (RMS) were significantly higher in NP-C than in controls in 3 out of 4 conditions. The Romberg quotient (RQ) to assess the amount of visual stabilization was significantly lower in the NP-C than in the HC group.
In contrast to other inherited metabolic disorders, such as Morbus Gaucher type 3, we did not find any evidence for an impairment of canal or otolith function in patients with NP-C as their cause of postural imbalance. Since RQ was low in NP-C patients, indicating proper sensory input, the observed increased postural sway is most likely due to a cerebellar dysfunction in NP-C, which may therefore explain postural imbalance.
INTRODUCTION

Niemann-Pick type C (NP-C) is a rare, multisystemic disease caused by pathological lipid storage and presenting with systemic, neurologic and psychiatric symptoms (Vanier, 2013). The cardinal symptom in all disease forms is vertical supranuclear saccade palsy, leading to complete gaze palsy in some patients, found in 70% of patients from an international disease registry (Patterson et al., 2013), but other ocular motor systems can also be impaired (Abel et al., 2009; Rottach et al., 1997). One of the most prominent neurologic symptoms, especially in juvenile and adult forms, is postural imbalance and gait disorder with recurrent falls. This may be due to impaired vestibular function, i.e. bilateral vestibulopathy (as in Morbus Gaucher type 3 (Chen et al., 2014) or chronic progressive external ophthalmoplegia (Ritchie et al., 2010)), cerebellar ataxia (Patterson et al., 2013) or a combination of both as in cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS) (Kirchner et al., 2011; Szmulewicz et al., 2011).

A vestibular deficit is plausible, because hearing is also impaired in patients with NP-C. A prior study showed high-frequency sensorineural hearing loss with retrocochlear involvement, with for hearing aids required at least in later stages of the disease (King et al., 2014) but, in the prior sibling studies, a normal vestibulo-ocular reflex (VOR) was observed (Lengyel et al., 1999; Solomon et al., 2005).

Intact function of the vestibular, proprioceptive and cerebellar systems is necessary for good balance and postural stability, which can be assessed by posturography (Krafczyk et al., 2006). With this tool, identification of the nature of the balance disturbance and topo-anatomical differentiation of the cerebellar impairment are possible (Diener et al., 1984).

The function of the vestibular system can be easily quantified nowadays. The angular VOR (aVOR) gain, defined as the ratio of eye velocity to head velocity, can be assessed by the video-based head impulse test (vHIT) in the high-frequency range (Agrawal et al., 2014) and by caloric irrigation in the low-frequency range (Halmagyi et al., 2000). Otolith function can be examined by ocular vestibular evoked myogenic potentials (oVEMP) for the utricle (Curthoys et al., 2012) and cervical VEMP for the saccule (Rosengren and Kingma, 2013). Furthermore, graviceptive pathways can be assessed by examination of the static subjective visual vertical (SVV) with the help of the bedside bucket test (Zwergal et al., 2009). In this study, we systematically examined the function of the vestibular system and postural balance in a cohort of eight patients with NP-C using the abovementioned clinical tools.
Vestibular function in NP-C

METHODS

Subjects. This study was conducted at a large tertiary outpatient clinic for vestibular and ocular motor disorders. Six patients with genetically confirmed and two patients with biochemically confirmed NP-C disease (2 females; age 27.3 ± 10.4 years (mean±SD), range 17-51 years, mean age of onset 9.4 ± 4.4 years, mean age at diagnosis 18.4 ± 13.8 years, mean disease duration 18 ± 12.4 years) were included. Results were compared with those of twenty age-matched healthy controls (HC) (11 females, 28.0 ± 10.9, range 11-57 years), with no history of vestibular, neuro-ophthalmologic or neurologic disease. Patients’ demographic and clinical characteristics are summarized in table 1. Clinical data of 5 out of 8 patients have been reported elsewhere (Bremova et al., 2015).

Table 1. Demographic and clinical characteristics of Niemann-Pick type C patients.

| Patient Nr/ | Sex/Age | Age of onset/diagnosis y | Medication | Genotype | Neurologic and psychiatric findings* | Internal manifestation/other findings | Tibial nerve SSEP/vibrationsNGS | Ocular motor findings | MRI findings | MoC | mDR | SAR |
|-------------|---------|--------------------------|------------|----------|--------------------------------------|---------------------------------------|-------------------------------|----------------------|--------------|------|------|-----|------|
| 1/M/24      | M/24    | 10/21/14                 | Miglustat 600 mg/d, hearing devices bilateral | NPC1: c.2474A>G, c.3160, c.3160G>A | b, c, d, f, h, k, l, m, s, t, y | Splenomegaly | Prolonged latencies L>R | Bilateral 8/8 | a, c, e | n | 25 | 8 | 11 |
| 2/M/20      | M/20    | 8/18/12                  | Miglustat 600 mg/d, risperidon 0.5 mg/d | No mutations in NPC1 and NPC2 genes found* | b, c, d, f, h, k, r, s, t | Mild hepatosplenomegaly | Prolonged latencies bilateral Radial 6/8 R, 5/8 L, malleolar r 6/8 R, 5/8 L | a, c, e | b | 23 | 7 | 10.5 |
| 3/F/51      | F/51    | 40/49/11                 | Miglustat 600 mg/d, hearing devices | NPC1: c.2621T>T, c.2872C>T | b, c, i, k, m, s, t, t | Mild splenomegaly | NP | a, b, c, e | a | NP | 6 | 4 |
| 4/M/28      | M/28    | 1/1/27                   | Miglustat 600 mg/d, | NPC1: c.3182T | b, c, d, f, s, t, y | Hepatosplenomegaly | N | a, c, h | a, c, d | 24 | 5 | 5 |
### Vestibular function in NP-C

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### Abbreviations: L = left, LE = lower extremities, MoCA = Montreal Cognitive Assessment, mDRS = modified Disability Rating Scale, MRI = Magnetic Resonance Imaging, N = normal, NP = not performed, R = right, SARA = Scale for the Assessment and Rating of Ataxia, SSEP = Somatosensory evoked potentials.

a Neurologic and psychiatric findings: a = epilepsy; b = ataxic stance and gait; c = dysmetria; d = dystonia; e = contractures of Achilles tendons; f = dysphagia; g = gelastic cataplexy; h =
Vestibular function in NP-C
dyskinesias; i = emotional instability/depression; j = clonus lower extremities; k = hyperreflexia; l = balbuties; m = hearing impairment; n = confined to a wheelchair; o = excessive salivation; p = hypomimia; r = organic psychosis; s = dysarthria; t = cognitive impairment; u = logorrhea; v = complete anarthria; x = developmental delay; y = tremor upper extremities; z = acatisia; aa = myoclonia; ab = hypofonia; ac = daily somnolence; ad = panic attacks; ae = verbal aggressiveness.

b Ocular motor findings: a = slow vertical saccades; b = vertical saccade paresis downward; c = impaired vertical smooth pursuit; d = fixation instability, e.g. square wave jerks; e = impaired vertical optokinetic nystagmus; f = strabism; g = slow horizontal saccades; h = impaired horizontal smooth pursuit.

c MRI findings: a = supratentorial enlarged liquor spaces; b = generalized atrophy; c = cerebellar atrophy; d = brainstem atrophy; e = leukodystrophy; f = periventricular signal enhancement; n = normal findings; g = calcifications.

d The total possible Montreal Cognitive Assessment (MoCA) score is 30 points; a score of 26 and higher is considered normal.

e The lowest possible modified Disability Rating Scale (mDRS) score is 0 points; the highest is 24.

f The lowest possible score of the Scale for the Assessment and Rating of Ataxia (SARA) is 0, the highest possible score is 40 points.

g Positive Filipin Staining (variant type).

Neurological examination. All patients received a thorough neurologic, neuro-ophthalmologic and neuro-otologic examination. To evaluate the overall status of NP-C disease, the modified Disability Rating Scale (mDRS) (Iturriaga et al., 2006; Pineda et al., 2009) was administered; cerebellar function was assessed by administration of the Scale for the Assessment and Rating of Ataxia (SARA) (Subramony, 2007; Weyer et al., 2007). Neurologic examination furthermore involved: examination of the cranial nerves, examination of the reflexes of the upper and lower extremities with pyramidal signs and extremity tonus. Proprioception was assessed clinically by tuning fork examination, with a normal finding rated as 6-8/8 and pathological proprioception rated as <5/8, and electrophysiologically by measuring somatosensory evoked potentials (SSEP) of the tibial and median nerves. Neuropsychologic status was assessed by the Montreal Cognitive Assessment (Nasreddine et al., 2005). All patients underwent brain magnetic resonance imaging (MRI) (see table 1).

Neuro-ophthalmologic and neuro-otologic examination comprised examination with and without Frenzel’s glasses to detect nystagmus, gaze-evoked nystagmus, head-shaking nystagmus, smooth pursuit, saccades, and optokinetic nystagmus.
Vestibular function in NP-C

**Ocular and cervical vestibular evoked myogenic potentials (o/cVEMP).** We used the same methodology as the one employed in previous studies (Agrawal *et al.*, 2013; Bremova *et al.*, 2013). For oVEMP, subjects lay in a supine position and were instructed to foveate a red dot fastened at the minishaker margin during oVEMP stimulation. Tap stimuli were delivered with a Bruel and Kjaer Mini-Shaker Type 4810 (2-ms clicks of positive polarity, with a repetition rate of 2 per second) at the midline of the hairline, 30% of the distance between the inion and nasion. The recording electrode was positioned over the inferior oblique muscle bilaterally, approximately 3 mm below the eye and centered beneath the pupil; a reference electrode was placed on the chin and a ground electrode under the chin. The responses to 50-100 stimuli were averaged. n1 and p1 were identified as the first negative and positive peaks that occurred between 10 and 20 ms after stimulus onset (Jongkees *et al.*, 1962). For cVEMP, participants were instructed to lift their heads with active straining of the sternocleidomastoid muscles on both sides to provide tonic background muscle activity during stimulation and recording. Air-conducted 500-Hz, 100-dB SPL tone bursts were delivered monaurally via intra-auricular headphones. Cervical VEMP were recorded from an electrode montage consisting of a recording electrode placed at the midpoint of the ipsilateral sternocleidomastoid muscle belly, a reference electrode placed on the manubrium sterni, and a ground electrode placed on the forehead. p1 and n2 peaks were identified as the first positive and negative peaks that occurred between 13 and 23 ms after stimulus onset. Peak-to-peak (PP) amplitudes, calculated as the sum of p1 and n2 peaks, were then divided by mean electromyographic activity recorded after the stimulus onset in order to check for background muscle activity. Corrected cVEMP PP amplitudes and p1 latencies were evaluated.

**Video-based head impulse testing (vHIT).** The aVOR gain (eye velocity/head velocity) (Aw *et al.*, 1996), assessed by the administration of the HIT with the EyeSeeCam system (Schneider *et al.*, 2009), was evaluated. Eye and head movements were recorded monocularly on the left eye with 2-dimensional video-oculography (VOG) (Bartl *et al.*, 2009). Each participant was instructed to binocularly foveate a visual target comprising a visual angle of 0.3° presented in the center of the 22” large computer monitor (LG, FLATRON W2242PK–SS, LG Electronics, Germany) running at 60 Hz. Monitor luminance was 250 cd/m². The monitor was positioned 60 cm from the participant’s nasion and subtended a visual angle of 43.2° horizontally by 27.7°.
Vertically. Eye dominance was not determined. The VOG system was calibrated for each participant by recording eye fixations at the central and eccentric positions aligned in 8.5° array across a range of ±15°. Calibration recordings were visually inspected to exclude artifacts. 10±2 head impulses were performed to each side.

**Caloric testing.** Caloric testing was performed with bithermal caloric irrigation (water temperature 30° and 44°, duration of irrigation 30 s). Patients lay in a supine position with their heads turned to the opposite ear during the measurement. Caloric-induced nystagmus was recorded for 2 minutes by means of the VOG system (EyeSeeCam® (Schneider *et al.*, 2009). To calculate the slow-phase velocity (SPV) of the caloric-induced nystagmus, eye velocity was calculated using numerical three-point differentiation of eye position and subsequent Gaussian low-pass filtering with a corner frequency of 30 Hz. The high-frequency velocity peaks of nystagmus quick phases, saccades and blink artifacts were removed from eye velocity using an absolute acceleration threshold and subsequent floating median filter with a time window of 0.25 s. For robust extreme value (maximum or minimum) determination, the squared fit was calculated from the SPV data window (±15 s about the extrema), which was previously filtered by a zero-phase digital filter. The peak slow-phase velocity (PSPV) of the caloric-induced nystagmus was calculated as an extreme value of the fitted curve.

All patients underwent caloric irrigation; the results of the caloric testing of 3 out of 8 patients (patients no. 3, 6 and 8) were not quantitatively analyzed due to non-compliance or artifacts. Directional preponderance was calculated using the standard Jongkees formula (Jongkees *et al.*, 1962) and PSPV values <5°/s were considered pathological. The asymmetry ratio was considered abnormal when ≥25%.

**Subjective visual vertical (SVV).** SVV was determined by binocular estimation of the dark straight line at the bottom of the bucket which was rotated clockwise or counterclockwise. A mean of 10 measurements, exceeding the range of values 0±2.3°, was considered a criterion for a pathological SVV tilt (Zwergal *et al.*, 2009).

**Posturography.** Posturographic examination was performed in the upright position with eyes open and closed, on firm ground (conditions 1 and 2) and on a slab of foam rubber (conditions 3 and 4) (Krafczyk *et al.*, 2006).
The total body sway (tSP) in 30 seconds of the posturographic measurement, expressed as the sway path values [m/min], root mean square (RMS) [mm] and frequency spectrum between 2.4-3.5 Hz (Fast Fourier Transform, FFT) of the z axis (head-vertical) (kgf/Hz) of the measurements were analyzed. The Romberg quotient (RQ), a ratio of the tSP with eyes closed and open, to assess the amount of visual stabilization, was calculated (Njiokiktjien and Van Parys, 1976).

**Statistical methods.** Statistical analysis and figure design were performed using SPSS version 22.0.0 (IBM, New York, NY, USA) and MATLAB (The Mathworks Inc). Differences were considered significant if $p < 0.05$. Normality of data distribution was tested using the mean, median, standard deviation and visual inspection of normal Q-Q plots and box plots. As data were not normally distributed, Wilcoxon related-samples rank test and non-related samples Mann-Whitney-U test were conducted. To assess the relationships between tested variables, Spearman rank correlation coefficient was used. Patients who were not physically capable of performing the particular score tasks or who did not perform the test for other reasons were excluded from the analysis.
RESULTS

Angular VOR. Representative raw data of a patient with NP-C and an HC subject are presented in figure 1A. The mean aVOR gain in patients with NP-C was 1.07 ± 0.12 and in controls 1.10 ± 0.12 (p = 0.469) (see figure 2A): There were no statistically significant relationships with other vestibular and neurologic tests (SVV: ρ = 0.250, p = 0.589; oVEMP n1 amplitude: ρ = 0.069, p = 0.727; cVEMP PP amplitude: ρ = 0.163, p = 0.408; SARA: ρ = -0.198, p = 0.670; mDRS: ρ = 0.146, p = 0.729). There was also no correlation with age (ρ = 0.157, p = 0.426), disease duration (ρ = 0.405, p = 0.320) or age of onset (ρ = 0.217, p = 0.606).

Figure 1. Angular vestibulo-ocular reflex, ocular and cervical vestibular evoked myogenic potentials in a patient with Niemann-Pick type C and a control. Representative raw traces of the angular vestibulo-ocular reflex (aVOR) (A), ocular and cervical vestibular evoked myogenic potentials (o- and cVEMP) (B) of a patient with Niemann-Pick type C (patient no. 5) and a healthy control. The blue line (1A) represents the mean of performed video-based head impulse tests (vHIT). The red line (1B) indicates the function of the otolith organs on the left side, the blue line of those on the right side.
**Caloric irrigation testing.** Mean PSPV of caloric-induced nystagmus was $18.5 \pm 6.8 \, ^\circ$/s in response to warm water and $12.6 \pm 5.3 \, ^\circ$/s in response to cold water. After excluding the patient who suffered a left labyrinth contusion with bleeding in 2008 after a bike accident with a left-side canal paresis with PSPV of $2^\circ$/s (patient no. 6), the asymmetry ratio was $10 \pm 1.6\%$.

**Ocular and cervical VEMP.** Representative raw data of the NP-C and HC subjects are presented in figure 1B. Mean oVEMP n1 amplitude was $11.5 \pm 5 \, \mu$V in NP-C and $12.0 \pm 4.8 \, \mu$V ($p = 0.784$) in HC groups. Mean oVEMP n1 latency was $12.1 \pm 1.5 \, ms$ in NP-C patients and $10.8 \pm 3.1 \, ms$ ($p = 0.199$) in HC subjects. Mean corrected cVEMP PP amplitude was $1.07 \pm 0.5 \, \mu$V in NP-C and $1.1 \pm 0.5 \, \mu$V in HCs ($p = 0.862$). Mean cVEMP p1 latency was $16.3 \pm 1.4 \, ms$ in NP-C and $16.6 \pm 1.4 \, ms$ in HC groups ($p = 0.980$). A graphical representation of the VEMP data is shown in figures 2B and 2C.

Correlation analysis also showed no significant relationships between age of onset (oVEMP n1 amplitude: $\rho = -0.639$, $p = 0.088$; n1 latencies: $\rho = -0.012$, $p = 0.977$; cVEMP PP amplitudes: $\rho = -0.566$, $p = 0.143$; p1 latencies: $\rho = 0.446$, $p = 0.268$) and o- and cVEMP amplitudes and latencies, as well as duration of disease (oVEMP n1 amplitudes: $\rho = 0.190$, $p = 0.651$; n1 latencies: $\rho = 0.262$, $p = 0.531$; cVEMP PP amplitudes: $\rho = 0.190$, $p = 0.651$; p1 latencies: $\rho = -0.238$, $p = 0.570$) and o- and cVEMP amplitudes and latencies.

There was no relation between neurological status, as assessed by mDRS and SARA and o-/cVEMP parameters in NP-C patients (mDRS: oVEMP n1 amplitude: $\rho = -0.586$, $p = 0.127$, n1 latency: $\rho = -0.073$, $p = 0.863$; cVEMP PP amplitudes: $\rho = -0.488$, $p = 0.220$, p1 latency: $\rho = 0.610$, $p = 0.108$; SARA: oVEMP n1 amplitude: $\rho = -0.419$, $p = 0.301$, n1 latency: $\rho = -0.299$, $p = 0.471$; cVEMP PP amplitudes: $\rho = -0.275$, $p = 0.509$, p1 latency: $\rho = 0.575$, $p = 0.136$).
Figure 2. Angular vestibulo-ocular reflex, ocular and cervical vestibular evoked myogenic potentials amplitudes and latencies in patients with Niemann-Pick type C and controls. Box plot representation of the angular vestibulo-ocular reflex (aVOR) gain (A), ocular vestibular evoked myogenic potentials (oVEMP) n1 amplitudes and n1 latencies respectively (B) and cervical VEMP peak-to-peak (PP) amplitudes and p1 latencies (cVEMP) (C) in patients with Niemann-Pick type C disease (NP-C) and healthy controls (HC). The light green depicts the VEMP amplitudes in HC, the dark green depicts the latencies in HC. The light blue depicts the VEMP amplitudes in NP-C and the dark blue depicts the VEMP latencies in NP-C. No statistically significant differences between the NP-C and HC groups were found for the vestibular measurements. The length of the boxes indicates the interquartile space (P25–P75); the horizontal line into the box represents the median (P50) and the whiskers indicate the adjacent values. The circles indicate the outliers.

Subjective visual vertical. SVV was tilted in 3 out of 8 patients with NP-C. The mean SVV was -0.18 ± 2.9 ((95% CI for the mean) -2.87 to +2.51) and was thus slightly higher than the range described previously (Zwergal et al., 2009). There was no significant relationship with any of the tests.

Posturography. Condition 1 (standing on firm ground with eyes open): The tSP was 1.8 ± 0.7 (1.2-2.4) m/min in NP-C patients (mean±SD, (95% CI for the mean)) and 0.7 ± 0.1 (0.7-0.8) m/min in controls (Z = -3.818, p = 4.5x10^-6) (difference: 1.1 m/min), the RMS was 12.5 ± 5.2 (7.7-17.3) mm in NP-C patients and 4.9 ± 1.9 (4.0-5.8) mm in controls (Z = -
3.375, \( p = 2.117 \times 10^{-4} \) (difference: 7.6 mm). The integral of the frequency spectrum (FFT Z) was 36.9 ± 41.6 (-1.6-75.4) kgf/Hz in NP-C patients and 7.3 ± 3.5 (5.6-8.9) kgf/Hz in controls (\( Z = -1.881, p = 0.06 \)).

**Condition 2** (standing on firm ground with eyes closed): The tSP was 1.8 ± 0.7 (1.1-2.4) m/min in NP-C patients and 0.9 ± 0.1 (0.8-1) m/min in controls (\( Z = -3.818, p = 4.504 \times 10^{-6} \)) (difference: 0.9 m/min), the RMS was 9.4 ± 5.1 (4.7-14.1) mm in NP-C patients and 5.2 ± 1.9 (4.0-6.1) mm in controls (\( Z = -2.434, p = 0.013 \)) (difference: 4.2 mm). The FFT Z was 19.4 ± 18.9 (1.9-36.9) kgf/Hz in NP-C patients and 6.1 ± 1.8 (5.3-7) kgf/Hz in controls (\( Z = -3.150, p = 0.002 \)).

**Condition 3** (standing on foam with eyes open): The tSP of NP-C patients yielded 3.8 ± 2.8 (1.3-6.4) m/min and tSP of controls 1.2 ± 0.3 (1.1-1.3) m/min (difference: 2.6 m/min) (\( Z = -2.43, p = 1.486 \times 10^{-4} \)). The RMS was 20 ± 6.6 (13.9-26.1) mm in NP-C patients and 8.3 ± 2.7 (7-9.6) mm in controls (difference: 11.7 mm) (\( Z = -3.486, p = 1.013 \times 10^{-4} \)). The FFT Z was 123.1 ± 131.5 (1.4-244.7) kgf/Hz in NP-C patients and 33 ± 12.8 (27-39) kgf/Hz in controls (\( Z = -1.771, p = 0.077 \)).

**Condition 4** (standing on foam with eyes closed): The tSP of NP-C patients yielded 4.8 ± 2.5 (2.5-7.2) m/min and in controls 3.3 ± 1 (2.8-3.7) m/min (difference: 1.5 m/min) (\( Z = -1.439, p = 0.162 \)). The RMS was 24.9 ± 7.6 (17.9-32) mm in NP-C and 19.6 ± 27 (16.2-22.9) mm in controls (difference: 5.5 mm) (\( Z = -1.605, p = 0.109 \)). The FFT Z was 132.8 ± 93.8 (46.1-219.5) kgf/Hz in NP-C patients and 98.2 ± 42.6 (77.6-118.7) kgf/Hz in controls (\( Z = -0.665, p = 0.506 \)).

The amount of visual stabilization, as assessed by the RQ on both firm ground and foam was significantly different across the groups, being higher in controls (firm ground: NP-C 1.01 ± 0.24 (0.8-1.2), HC 1.27 ± 0.25 (1.2-1.4) \( p = 0.022 \); foam: NP-C 1.4 ± 0.6 (0.9-1.9), HC 2.7 ± 0.8 (2.4-3.1), \( p = 2.117 \times 10^{-4} \)).

In one patient, discrete cerebellar 3 Hz postural sway was seen (patient no. 4 in table 1). This finding has a morphological correlate in a cerebellar atrophy, as seen in MRI.

The frequency plot of an NP-C patient, a patient with a cerebellar 3-Hz sway and a healthy subject is shown in figure 3A, and a graphical representation of the tSP and RMS values is presented in figure 3B.
Vestibular function in NP-C

Figure 3. Posturographic results in patients with Niemann-Pick type C and healthy controls. Frequency plot of the z axis (kgf/Hz) in a patient with Niemann-Pick type C (NP-C) (blue line), a patient with cerebellar sway (red line) and a normal subject (green line) standing on foam with eyes closed (3A). Note that the frequency pattern of the NP-C patient is not significantly different from that of a healthy subject with a normal frequency distribution. Bar representation of the differences in total sway path (tSP) and root mean square (RMS) values in patients with Niemann-Pick type C and healthy controls (3B).

* indicates significant difference at the 0.05 level
** indicate significant difference at the 0.001 level

Correlation analyses. There was no significant association between neurologic status, as assessed by mDRS and SARA, and posturographic parameters in NP-C patients (mDRS: tSP in condition 1: $\rho = 0.296, p = 0.518$, RMS: $\rho = 0.074, p = 0.875$; tSP in condition 2: $\rho = 0.222, p = 0.632$; RMS: $\rho = 0.667, p = 0.102$, tSP in condition 3: $\rho = -0.037, p = 0.937$, RMS: $\rho = 0.259, p = 0.574$; tSP in condition 4: $\rho = -0.074, p = 0.875$, RMS: $\rho = 0.185, p = 0.691$; SARA: tSP in condition 1 $\rho = 0.559, p = 0.192$, RMS: $\rho = 0.721, p = 0.068$; tSP in condition 2: $\rho = 0.126, p = 0.788$; RMS: $\rho = 0.631, p = 0.129$, tSP in condition 3 $\rho = -0.180, p = 0.699$, RMS: $\rho = 0.487, p = 0.268$; tSP in condition 4: $\rho = -0.595, p = 0.159$, RMS: $\rho = 0.523, p = 0.229$).

No significant relationships between the posturographic and VEMP parameters, but one (tSP value in condition 4 and cVEMP PP amplitudes) were seen (oVEMP n1 amplitude: tSP in condition 1: $\rho = 0.170, p = 0.397$, RMS: $\rho = 0.192, p = 0.338$; tSP in condition 2: $\rho = -0.036, p = 0.858$; RMS: $\rho = -0.125, p = 0.536$, tSP in condition 3: $\rho = 0.148, p = 0.462$, RMS: $\rho = 0.191, p = 0.340$; tSP in condition 4: $\rho = 0.001, p = 0.998$, RMS: $\rho = 0.155, p = 0.440$; cVEMP PP amplitudes: tSP in condition 1: $\rho = -0.001, p = 0.998$, RMS: $\rho = 0.018, p = 0.930$; tSP in condition 2: $\rho = -0.150, p = 0.455$; RMS: $\rho = -0.270, p = 0.172$, tSP in condition 3: $\rho = 0.015, p =$
0.940, RMS: ρ = 0.051, p = 0.799; tSP in condition 4: ρ = -0.384, p = 0.048, RMS: ρ = -0.281, p = 0.155).
Table 2. Posturographic results in Niemann-Pick type C patients and healthy controls.

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**Abbreviations:** HC = healthy controls, IQR = interquartile range, FFT Z = Fast Fourier Transform of the z axis, FS = frequency spectrum, RQ = Romberg quotient, tSP = total Sway Path, RMS = Root Mean Square.

\( a \) Parameters are depicted as mean (standard deviation) and median (interquartile range), \( b \) All patients, but one (patient no. 7) underwent posturographic measurements, \( c \) Standing on firm ground with eyes open, \( d \) Standing on firm ground with eyes closed, \( e \) Standing on foam with eyes open, \( f \) Standing on foam with eyes closed, \( g \) Romberg quotient (RQ)- a ratio of the total sway path with eyes closed to eyes open.
Discussion

The major findings of this study are as follows: first, we did not find any evidence for an impairment of angular VOR function (in either the high-frequency or in the low-frequency range) or otolith function (in either the utricle or the saccule); second, patients showed remarkable postural instability compared with normal subjects; third, analysis of the posturographic findings indicated diffuse cerebellar disturbance with potential involvement of the vestibulo-cerebellum. Based on our results, vestibular horizontal canal and otolith function is intact.

These findings were unexpected in light of the fact that the vestibular system is commonly affected in neurodegenerative disorders (Shaikh et al., 2011; Strupp et al., 2014). In fact, in another lysosomal storage disease, Gaucher disease type 3 (neuronopathic type, GD3), impaired otolith pathways and aVOR deficits with absent horizontalrefixation saccades were described (Chen et al., 2014). This might be explained by the neuronal loss and functional disturbance of the vestibular nuclei in the medulla and paramedian pontine reticular formation (PPRF) respectively, leading to horizontal saccade palsy in GD3 disease. Vestibular nuclei and their afferent and efferent projections seem to be functionally intact in NP-C disease. The specific pattern of impairment in NP-C and GD3 diseases suggests a different neuronal susceptibility to the toxic effects of the storage material. The intact vestibular organs are also reflected in a lack of correlation between any of the vestibular tests and clinical rating scales.

In terms of posturography, we found that patients with NP-C had increased body sway compared with controls in 3 out of 4 conditions. Disturbance of the somatosensory input in condition 4 led to an equal increase of the total sway path in both groups. The effect of vision was significantly more pronounced in controls, since the Romberg quotient was significantly higher in controls than in patients with NP-C. This finding also indicates the functionally intact vestibular organs, as visual cue is known to be of high importance in peripheral vestibulopathy to compensate for the vestibular loss (Krafczyk et al., 2006; Nashner et al., 1982). Moreover, due to the saccadic deficits, motor performance and orientation in space that require visual-vestibular interaction in patients with NP-C are impaired. It is likely that the balance network compensates for these ocular deficits by enhancing the other sensory input, especially vestibular and somatosensory input. Balance is not based on a fixed set of equilibrium reflexes but on a flexible, functional motor skill that can adapt with training and experience and presumably reflects noise and regulatory activity within afferent-efferent control loops, which are plastic enough to compensate for the existing deficits.
Visual stabilization had no remarkable influence on the postural stability, as indicated by the low Romberg quotient of the total sway path in patients with NP-C, even though the proprioception was diminished in 3 patients. A low proportion of visual stabilization suggests that the spino-cerebellum and its spinal afferents are not primarily affected by the disease process. Nevertheless, atrophy of the cerebellar vermis in severe cases of NP-C disease has been previously described (Fusco et al., 2012; Huang et al., 2011).

In the frequency analysis, there was no consistent 3 Hz anteroposterior cerebellar sway in patients with NP-C. One patient with cerebellar atrophy in MRI had increased postural sway at 2-3 Hz frequency, but he did not reach values seen in patients with anterior lobe lesions (Diener et al., 1984).

All in all, the constellation of the posturographic findings (pathological sway parameters with poor visual stabilization without 3 Hz sway) suggests a rather diffuse cerebellar disturbance with a possible involvement of the vestibulo-cerebellum, rather than isolated impairment of the spino-cerebellum, anterior lobe or cerebellar hemispheres (Diener et al., 1984; Schwesig et al., 2009). In contrast, a patterned degeneration of the Purkinje cells from anterior to posterior, with surviving Purkinje cells in lobules IX and X at the terminal stages of the disease has been described in an NP-C mouse model (Sarna et al., 2003). Our NP-C cohort was relatively young (mean age = 27.3 years, mean SD = 10.4 years) with a rather mild cerebellar disturbance (mean SARA score 9.3/40) and lacking severe involvement of any circumscribed region of the cerebellum. Thus, the pattern of neurodegeneration described in the mouse model could not be observed.

The isolated cerebellar impairment without vestibular involvement is also in line with previously shown increased metabolism in the vestibulo-cerebellum in a rat model of peripheral vestibulopathy under therapy with acetyl-DL-leucine (Günther et al., 2015). The beneficial effect of this therapy was seen recently in a cohort of 12 NP-C patients (Bremova et al., 2015), improving stance and gait, diadochokinesis and diminishing the intensity of the square wave jerks, indicating a stabilizing effect on impaired cerebellar Purkinje cells, similar to the effect on neurons of medial vestibular nucleus in an unilateral-vestibular-loss guinea-pig model (Vibert and Vidal, 2001b). This study has some limitations. First, the sample size is small, given that the NP-C disease is an orphan disease, meaning that this study might be underpowered. Second, as patients become fatigued very quickly, some tests were not performed due to the lack of compliance or due to physical disability or cognitive impairment. Third, there is an ongoing
discussion about the sensitivity and specificity of VEMP investigation, principally because of its high interindividual, but also interrater variability (Ertl et al., 2015).

ETHICAL STANDARDS
The study was performed in accordance with the Helsinki II Declaration and was approved by the ethics committee of the Ludwig-Maximilians University Medical Faculty (No. 379-12). All participants gave their informed consent prior to their inclusion in the study.

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DISCLOSURES
J.R. received speaker’s honoraria from BioMarin, Shire, Genzyme and Actelion. M.S. is Joint Editor-in-Chief of the Journal of Neurology, Editor-in-Chief of Frontiers of Neuro-otology and Section Editor of F1000. He has received speaker’s honoraria from Abbott, Actelion, UCB, GSK, TEVA, Heel, Biogen, Pierre-Fabre, Eisai and Hennig Pharma. He also works as a consultant for Abbott, Heel, Synthon and Actelion.
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Vestibular function in NP-C


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Discussion

Within the framework of this thesis, two aspects of NP-C disease have been investigated. First, we evaluated the effect of treatment with the amino-acid acetyl-DL-leucine on cerebellar symptoms, eye movements and quality of life. Second, we evaluated the function of the vestibular system in patients with NP-C versus healthy controls, and its contribution to postural imbalance, including topo-anatomical assessment of the quantitative posturographic measures. For these two studies, patients with NP-C had to be recruited from 3 European countries (Germany, Slovakia, Czech Republic) and were systematically examined.

A plethora of techniques were used. Patients were assessed clinically by means of clinical rating scales (Scale for the Assessment and Rating of Ataxia, the Spinocerebellar Ataxia Functional Index, modified Disability Rating Scale, Montreal Cognitive Assessment, ED-5Q-5L Quality of Life with visual analog scale). Further, different types of eye movements were examined video-oculographically (saccades, smooth pursuit, gaze-holding, optokinetic nystagmus in horizontal and vertical planes), and video-head impulse test to measure the function of the high-frequency angular vestibulo-ocular reflex. And last, but not least, cervical and ocular vestibular evoked myogenic potentials to measure the function of the saccule and utricle, as well as posturography with a neuronal network analysis to measure postural imbalance and the underlying cause, were applied.

1.3 Effects of the therapy with acetyl-DL-leucine in patients with NP-C disease

We treated a cohort of 12 patients with NP-C disease with amino-acid acetyl-DL-leucine for 4 weeks. We were able to show that: first, the modified amino-acid acetyl-DL-leucine had a significant effect on cerebellar signs and symptoms in patients with genetically and/or biochemically proven NP-C disease. Second, the improvement of neurologic status also led to a significant improvement of the quality of life of the patients and their family members. Third, the therapy also improved the cognitive function and functioning in daily life, in terms of a slight stabilization of emotional affect, an increase in drive and social interaction, as well as improved performance of complex tasks, such as dressing or drawing. Fourth, the low frequency (1 of 12) and the temporary nature of adverse effects suggested a reasonable risk-benefit profile.
The limitations of this study were a) a lack of a reference agent (placebo), b) the non-blinded design, c) small sample size and the short observational period. Thus, in a future project, we aim to prove the effect of medication under these conditions (see the section 1.4.1.2).

Current state of knowledge indicates that acetyl-DL-leucine restores the normal membrane potentials of both hyper- and depolarized neurons (Vibert and Vidal, 2001a). Since there is evidence for calcium dyshomeostasis in NP-C disease, within particular functionally disturbed Purkinje cells, similar to SCAs, restoring the calcium equilibrium within the cerebellum might be the actual mode of action in NP-C.

This is supported by the fact that we found no evidence for a vestibular disturbance in patients with NP-C in our second study. We hypothesize that the effect of the therapy is probably due to the direct action on Purkinje cells in the cerebellum, rather than on the intact vestibular centers in the brainstem.

All in all further studies focusing on the action of acetyl-DL-leucine in both humans and animal models are a high priority.

1.4 Vestibular function and postural balance in patients with NP-C disease

In the second study, we were able to show that horizontal aVOR and otolith functions are, in contrast to other lysosomal storage disorders such as GD3, intact in patients with NP-C. This was surprising in light of the documented deficits in GD3 disease which exhibits decreased VOR gain, increased latency and absence of refixation saccades (Chen et al., 2014).

Moreover, saccular function was partially diminished and utricular function was fully diminished in GD3 patients, which was also not the case in patients with NP-C. This indicates that even though the origin of both disorders is a well-known failure of the degradation of lysosomal material, the way lipid storage causes the pattern of neurodegeneration seen in NP-C and GD3 is still not fully understood.

A Fourier analysis of posturographic data revealed that the stance and gait unsteadiness is indeed of cerebellar origin, with possible involvement of the vestibulo-cerebellum (flocculonodular lobe), rather than isolated impairment of the spino-cerebellum, vermis or cerebellar hemispheres, even though proprioception was diminished in some patients. There was also no characteristic 3 Hz cerebellar sway, seen in patients with vermis atrophy (Sullivan et al., 2010). Since our NP-C cohort was relatively young (mean age = 27.3 years, SD = 10.4 years), there was no severe involvement of any particular cerebellar region, underlying high-grade ataxia (mean
SARA score 9.3/40). This might be biased by patient selection, since the severely affected palliative patients were not able to participate in the study, due to the motor, but also cognitive deficits. Thus, we did not observe the common pattern of neurodegeneration, beginning in lobules I and II and progressing from anterior to posterior, relatively sparing the vestibulocerebellum described in the mouse model of NP-C disease (Sarna et al., 2003).

Although the patients with NP-C disease present hearing and ocular motor deficits, arising from the specific impairment of the cochlear and vertical saccadic centers in the brainstem, based on our results the central and peripheral vestibular centers are intact. From a clinical perspective, the saccadic system, which reflects the disease dynamics, rather than the vestibular system, can be used to track the disease progress and therapy effect at patients` follow-up.

1.4.1 Further investigations, work in progress and perspectives in NP-C disease

1.4.1.1 Systematic and standardized explorative, multicenter, observational ocular motor study

The hallmark and initial symptom of NP-C disease is the vertical supranuclear saccade palsy (VSSP). In the course of disease, smooth pursuit also deteriorates, leading to a gaze restriction and a vertical supranuclear gaze palsy (VSGP). The VSGP has been found in 70% of patients in an international patient registry (Patterson et al., 2013). Nevertheless, to detect the VSSP, both saccades and smooth pursuit have to be examined. This depends on the clinical expertise, so that an actual prevalence of the VSSP in patients with NP-C might be considerably higher, based on our expert experience possibly yielding 95%.

The saccadic function in NP-C disease has been investigated in previous works (Abel et al., 2009; Lengyel et al., 1999; Solomon et al., 2008), nevertheless, these studies suffer from limitations. First, given the rarity of the disease studies often have a small number of participants, and thus these studies might be underpowered. Second, the course of the disease is highly variable, so that generalisations about ocular motor findings across the NP-C population based on the observations in siblings might lack validity. Third, eye movement examinations might be reduced in quality with some older eye-tracking systems (such as infra-red systems), so that new studies using new video-oculographic tools are required to re-evaluate the already existing findings.

Thus, we are currently analysing ocular motor data of the largest prospectively examined NP-C cohort of 31 NP-C patients, measured across four European countries (Germany, Italy,
Czech Republic, Slovak Republic) by means of the video-oculographic tool (EyeSeeCam®) (Schneider et al., 2009). Given its non-invasivity, simplicity and time-efficiency, which considerably increase the patient compliance, children could also be measured (Figure 6). Horizontal and vertical saccades, smooth pursuit, gaze-holding, optokinetic nystagmus and horizontal angular VOR were examined. Moreover, in order to relate and compare the ocular motor impairment with the clinical status, clinical rating scales, including mDRS, SARA, SCAFI and MoCA were administered.

**Figure 6.** Mobile video-oculographic set-up allows non-invasive measurement of pediatric patients.

This study has a considerable impact in terms of future diagnostic and screening studies. On the one hand, it has identified the particular challenges and confounding factors of this examination in a large, genetically heterogeneous cohort of pediatric and adult patients with NP-C. The examination is challenging both for the considerable ocular motor deficits found in these patients (gaze palsy), their compensation strategies (blinking, head movements), but also because of their sometimes very restricted cognitive abilities. Since the problems seen in the video-oculographic examination can be observed also in other neurodegenerative disorders such as...
Gaucher disease type 3 or ataxia telangiectasia, this set-up can be used broadly in a clinical routine, meaning that the data collected multicentrically is consistent and of minimal variability. On the other hand, since it is non-invasive, it can also be used to screen for patients with neurodegenerative disorders, who initially demonstrate subtle ocular motor changes, such as slow vertical saccades in NP-C. This is of great importance, especially in treatable metabolic diseases, where establishing a diagnosis implies the administration of a disease-specific therapy, such as miglustat in NP-C, which has been shown to at least slow the progression of the disease and thus, prolong the life expectancy of these patients (Patterson M et al., 2015).

On top of the abovementioned benefits of this pilot examination, we aim to: first, characterize the ocular motor systems more in detail, describing the so-called saccadic patterns. Second, establish the link between the saccadic patterns and clinical phenotype. Third, correlate the quantitative ocular motor parameters and patterns with the phenotype assessed by the clinical rating scales to find potential ocular motor biomarkers that may serve as clinical outcome measures for future clinical trials.

1.4.1.2 Explorative, multicenter, cross-sectional longitudinal ocular motor study in patients with NP-C

In addition to the abovementioned study, a longitudinal standardized and systematic ocular motor study to assess the progress of disease and response to treatment, is currently in preparation. A cohort of 50 to 100 patients from Germany, France, Czech Republic, Italy, Spain, Russia, Brazil and Iran, together with the same number of gender and age-matched controls will be ocular motor and neurologically examined. Horizontal and vertical reflexive and self-paced saccades will be used to evaluate the function of the supranuclear brainstem structures (riMLF and PPRF) together with cortical areas for saccade generation (Frontal Eye Field, FEF for voluntary saccades and Pariental Eye Field, PEF for reflexive saccades). Moreover, gaze-holding in eccentric positions to evaluate the function of cerebellar flocculo-nodular lobe and supranuclear brainstem structures (Ncl. interstitialis Cajal, INC for vertical and Nucleus praepositus hypoglossi, NPH for horizontal gaze-holding), as well as smooth pursuit to evaluate the function of cerebellum and pontine nuclei, will be performed. To compare the ocular motor systems with clinical status, mDRS will be administered and subsequently correlated with a battery of ocular motor parameters, including saccadic peak velocity, mean velocity, amplitude, gain, latency, acceleration, peak duration,
mean duration as well as the slopes of linear regression line of the analyzed parameters, smooth pursuit gain, slow-phase velocity of gaze-holding nystagmus (if present).

1.4.1.3 Therapy of lysosomal storage disorders (Niemann-Pick disease type C and Gaucher disease type 3) with acetyl-DL-leucine: Randomized, double-blind, placebo-controlled, 2 way cross-over phase II NGAT trial

Based on the initial studies with acetyl-DL-leucine for treatment of cerebellar ataxia in patients with NP-C and degenerative cerebellar ataxia syndromes, we are currently planning a randomized, double-blind, placebo-controlled, 2 way cross-over phase II trial. The added value of these clinical studies is the demonstrated safety and tolerability of the agent in different medical conditions with a common symptom of cerebellar ataxia. As mentioned above, the limitations of these studies are a) a lack of the reference agent (placebo), b) the non-blinded design, c) small sample size and the short observational period. Thus, this project aims to prove the effect of medication under these conditions in patients with NP-C and GD3.

Although miglustat helps to stabilize the neurological involvement in NP-C (Patterson et al., 2015), evidence that miglustat stabilizes the cerebellar ataxia is still lacking. Furthermore, enzyme replacement therapy (ERT) ameliorates the systemic manifestations, but has no effect on the neurological involvement in GD3, stressing the importance of alternative therapies in this disorder (Vellodi et al., 2009).

In this study, we aim to evaluate the efficacy of acetyl-DL-leucine in patients with genetically and/or biochemically diagnosed NP-C and GD3 older than 5 years of age with SARA more than 2 points. A reference intervention will be treatment with placebo in double-blind fashion (for study design, also see Figure no. 7). Duration of intervention per patient will be sixteen weeks in total, including 2x4 weeks of verum/placebo in cross-over design, 4 weeks wash-out between both treatment periods and follow-up visit after 4 weeks.
1.4.1.4 Clarifying the action of acetyl-DL-leucine in vivo and in vitro

Our clinical study to evaluate the effects of the therapy with acetyl-DL-leucine in patients with NP-C has demarcated some emerging implications in basic science research, especially in the field of cell biology.

Calcium dyshomeostasis in the late endosome/lysosome occurs in NP-C disease, establishing a link between NP-C disease and spinocerebellar ataxias (SCAs) (Lloyd-Evans et al., 2008).

To date, the lysosomal cell biology group around Dr. Lloyd-Evans at Cardiff University, UK has identified that there is a role for changes in Ca\(^{2+}\) signalling as one potential mechanism explaining how acetyl-DL-leucine works in NP-C disease cells. There is evidence that acetyl-DL-leucine acts via an extracellular Ca\(^{2+}\) sensing receptor (CaSR), which preferentially binds positively charged L-configurated amino-acids. Our collaborators in Cardiff observed that there is a reduction in storage in NP-C cells treated with acetyl-DL-leucine and that if the Ca\(^{2+}\) concentration is changed, no benefit in the NP-C cells can be observed. Moreover, they have also found that this benefit correlates with expression of CaSR.

Furthermore, calbindin is a Ca\(^{2+}\) binding protein and a useful marker in NP-C disease as in NPC1 it is secreted in high levels into the CSF and plasma, thus reflecting the changes in Ca\(^{2+}\) that occur in NP-C cells. If acetyl-DL-leucine changes intracellular Ca\(^{2+}\) levels via CaSR
regulation then there might be changes in the levels of calbindin in plasma, confirmed by ELISA. For the lipids, if acetyl-DL-leucine is altering Ca\textsuperscript{2+} levels then there might be some normalisation of lipid storage, which can be confirmed by the lipidomics.

The aim of this work in progress is to evaluate whether the reduced lipid levels in the patient samples (and changes in the Ca\textsuperscript{2+} binding protein calbindin) are in line with the cell culture findings, providing clinical reference for the mechanism found. This might also clarify how acetyl-DL-leucine works for other disorders such as SCA, since by altering Ca\textsuperscript{2+} sensing receptor function, cellular Ca\textsuperscript{2+} levels will change.

The changes in Ca\textsuperscript{2+}, CaSR and calbindin can be subsequently correlated with the changes in the clinical rating scales before and after therapy with acetyl-DL-leucine.
1.5 Conclusion

We showed in an observational study that a therapy with the modified amino acid acetyl-DL-leucine improves cerebellar ataxia and quality of life in patients with NP-C disease. These findings are the basis for a planned multinational randomized, placebo-controlled, double-blind, 2-way cross-over phase II trial in patients with NP-C and GD3. Further, the therapeutic effect of acetyl-DL-leucine seen in our case series stimulated collaboration with colleagues UK who are now looking for its binding sites, changes of calcium homeostasis and lipid storage as well as its impact on ion channels. These findings will also have implications for the explanation of the pathophysiology of NP-C.

We demonstrated that – in contrast to other inherited metabolic disorders such as GD3 – there is no evidence of an impairment of the vestibular system in patients with NP-C, neither for canal nor for otolith function. Postural imbalance in these subjects is therefore mainly due to cerebellar dysfunction, which is supported by frequency analyses of posturographic data from these patients.

In a cross-sectional study on the ocular motor system in patients with NP-C using a mobile video-oculographic tool, we identified different types of impairments of eye movements. These findings are the basis for large international cohort studies of pediatric and adult patients with NP-C. In these studies, the impairments of eye movements are correlated with other scales and are used as biomarker for the progression of the disease and the effects of therapy.
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### Curriculum vitae

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- Clinical trials on the pharmacotherapy of cerebellar, ocular motor and neurodegenerative disorders  
- Otolith function in peripheral and central vestibular and cerebellar disorders, such as benign paroxysmal positional vertigo and downbeat nystagmus syndrome

### Clinical trial doctor in:
- **BEMED:** Effect of treatment with betahistine in Menière´s disease  
- **BETAVEST:** Effect of treatment with betahistine in vestibular neuritis
Curriculum vitae

- **PROVEMIG**: Effect of treatment with metoprolol in vestibular migraine

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- Hands-on trainings on the standardized and systematic vestibular and ocular motor examination
- Hand-on trainings on mobile video-oculography
- Talks on differential diagnosis of the central and peripheral vestibular and ocular motor disorders at a number of national and international congresses and meetings, inclusive annual Vertigo Congress of the German Center for Vertigo and Balance Disorders at the LMU Munich, Campus Großhadern
Current publications


I hereby confirm that the dissertation „Niemann-Pick type C disease: Effects of a therapy with acetyl-DL-leucine and vestibular function.“ is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

München, den/ Munich, date

Unterschrift/ signature
Author contributions

The authors contributed to the publications, as follows:

1. Bremova T*, Malinová V, Amraoui Y, Mengel E, Reinke J, Kolníková M, Strupp M. **Acetyl-dl-leucine in Niemann-Pick type C: A case series.** TB: design of the study, conducting experiments, data analysis, data interpretation, drafting/revising the manuscript. VM: patient recruitment/data interpretation/revising the manuscript for important intellectual content. YA: patient recruitment/revising the manuscript for important intellectual content. EM: revising the manuscript for important intellectual content. JR: revising the manuscript for important intellectual content. MK: patient recruitment/revising the manuscript for important intellectual content. MS: study concept/revising the manuscript for important intellectual content.

2. Bremova T*, Krafczyk S., Bardins S, Reinke J, Strupp M. **Vestibular function in patients with Niemann-Pick type C disease.** TB: idea for the study, design, data collection, data interpretation, statistical analysis, writing the manuscript. SK: data interpretation, revising the manuscript for important intellectual content. SB: figure design, revising the manuscript for important intellectual content. JR: revising the manuscript for important intellectual content. MS: revising the manuscript for important intellectual content.

I thereby certify that the abovementioned statements in regards to the author contributions are correct.

Place and date

__________________________  __________________________
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