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Elevated Cerebrospinal Fluid Ubiquitin C-terminal Hydrolase-L1 Levels Correlate with Phenotypic Severity and Therapeutic Response in Niemann-Pick Disease, type C1

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NXC	Performed assays; Supervision; Edited manuscript.
SG	Performed statistical evaluation and Drafted Statistical Report.
NMF	Maintained Clinical Protocol; Clinical evaluations; Biospecimen Collection; Clinical Data Collection and Organization; Supervision.
RAL	Performed assays.
KEJS	Performed assays.
HOM	Clinical data collection and organization.
ADD	Conceptualization, Clinical evaluations; Biospecimen Collection; Edited Manuscript.
EB-K	Conceptualization and funding.
SMC	Conceptualization and funding.
FL	Performed statistical evaluation; Funding; Supervision; Edited Manuscript.
FDP	Conceptualization, Maintained Clinical Protocols; Clinical Evaluations; Biospecimen Collection; Supervision; Funding; Wrote Manuscript

Study Approval

The CSF and clinical data used in this study were obtained as part of natural history/observational trials initially approved by the NICHD IRB. Ongoing IRB approval is by the NIH Clinical Center IRB.

Declaration of Competing Interest None

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Abstract

Background: Niemann-Pick disease, type C1 (NPC1) is an ultrarare, recessive disorder due to pathological variants of *NPC1*. The NPC1 phenotype is characterized by progressive cerebellar ataxia and cognitive impairment. Although classically a childhood/adolescent disease, NPC1 is heterogeneous with respect to the age of onset of neurological signs and symptoms. While miglustat has shown to be clinically effective, there are currently no FDA approved drugs to treat NPC1. Identification and characterization of biomarkers may provide tools to facilitate therapeutic trials. Ubiquitin C-terminal hydrolase-L1 (UCHL1) is a protein which is highly expressed by neurons and is a biomarker of neuronal damage. We thus measured cerebrospinal fluid (CSF) levels of UCHL1 in individuals with NPC1.

Methods: CSF levels of UCHL1 were measured using a Quanterix Neuroplex 4 assay in 94 individuals with NPC1 and 35 age-appropriate comparison samples. Cross-sectional and longitudinal CSF UCHL1 levels were then evaluated for correlation with phenotypic measures and treatment status.

Results: CSF UCHL1 levels were markedly elevated (3.3-fold) in individuals with NPC1 relative to comparison samples. The CSF UCHL1 levels showed statistically significant (adj $p<0.0001$), moderate, positive correlations with both the 17- and 5-domain NPC Neurological Severity Scores and the Annual Severity Increment Scores. Miglustat treatment significantly decreased (adj $p<0.0001$) CSF UCHL1 levels by 30% (95% CI 17-40%).

Conclusions: CSF UCHL1 levels are elevated in NPC1, increase with increasing clinical severity and decrease in response to therapy with miglustat. Based on these data, UCHL1 may be a useful biomarker to monitor disease progression and therapeutic response in individuals with NPC1.

Keywords

Niemann-Pick disease; type C1; NPC1; Ubiquitin C-terminal hydrolase-L1; UCHL1; Biomarker; Miglustat

1. Introduction

Identification of biomarkers can provide insight into disease mechanisms and provide tools to support development of therapeutic interventions. Acceptance of disease relevant biomarkers by regulatory agencies is key to defining a path forward for ultrarare disorders where a conventional clinical trial design is confounded by very low number of eligible participants, phenotypic heterogeneity, and slow disease progression. Use of biomarkers for accelerated approval was critical to the rapid development of effective combination therapies for treatment of HIV and the concept needs to be applied to ultrarare disorders (1, 2). Disease related biomarkers may also prove useful in prognostic counseling and monitoring disease progression.

Niemann-Pick disease, type C1 (NPC1) is a very rare genetic disorder with an incidence of the classical childhood/adolescent disease estimated to be on the order of 1/100,000 (3, 4). The Niemann-Pick type C (NPC) histopathology and phenotype were well described by Crocker and Farber in 1958 (5). NPC1 is an autosomal recessive disorder due to pathological variants of *NPC1*. A very similar, but much rarer (incidence ~ 1/2,000,000), disorder is due to pathological variants of *NPC2*. The NPC1 phenotype is heterogeneous, both with respect to variable disease signs/symptoms and age of neurological onset. Somatic disease is also variable. Some infants have mild cholestatic jaundice with hepatosplenomegaly, while others can progress to liver failure. Neurological signs/symptoms are primarily characterized by progressive cognitive impairment and cerebellar ataxia. Vertical supranuclear gaze palsy is an early and near universal neurological finding. Other neurological, but variable, issues include seizures and gelastic cataplexy. Swallowing dysfunction is common, with complications from aspiration being a leading cause of death in this patient population (3, 6–9).

NPC1 is a lysosomal disorder. The NPC1 protein, in concert with NPC2, functions to transport unesterified cholesterol from the lysosomal lumen and make it bioavailable to support cellular functions (10). A deficiency of NPC1 function leads to the endolysosomal accumulation of unesterified cholesterol and corresponding deficiency of bioavailable cellular cholesterol. This leads in turn to neurological dysfunction and neuronal loss. Although all neurons are affected, some neurons appear to be more sensitive to the loss of NPC1 function. Loss or dysfunction of neurons in the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) is the cause of the characteristic vertical supranuclear gaze palsy, whereas loss of cerebellar Purkinje neurons underlies the cerebellar ataxia.

There are no FDA approved therapies for NPC1. Miglustat, a glycosphingolipid synthesis inhibitor, has been approved for the treatment of NPC1 disease by regulatory agencies in most other countries. Miglustat, however, is approved in the United States for the treatment of Gaucher disease, thus it is available off-label. Its use to treat NPC1 is supported by real-life data showing that it slows the rate of swallowing dysfunction and increases survival (8, 9, 11–14). However, in a phenotypically heterogeneous disease that progresses over years, it is not feasible to reliably collect clinical efficacy data in short, controlled trials. This issue is illustrated by failure of the initial 12-month miglustat study (13) to obtain FDA approval as well as failure, thus far, of both a 12-month sham controlled trial of intrathecal 2-hydroxypropyl-β-cyclodextrin (adrabetadex, NCT0253844) and a 12-month placebo-controlled trial of arimoclomol (NCT02612129, (15)). Identification of disease relevant biomarkers that reflect clinical disease status and respond to therapeutic interventions could be useful in supporting development of therapies for this progressive neurodegenerative disease where classical placebo-controlled trials are not adequate. Recently, the USA Federal Drug Administration approved tofersen for a rare form of amyotrophic lateral sclerosis caused by pathological variants of SOD1 based on a reduction of serum neurofilament light levels (16). The accelerated approval pathway allows for the approval of a drug for a serious illness when a biomarker is reasonably likely to predict a clinical benefit.

Multiple CSF proteins have been evaluated as biomarkers for common neurological diseases and neuronal injury. This includes UCHL1, or ubiquitin C-terminal hydrolase-L1, a protein that is highly expressed in neurons. UCHL1 has been reported to be a biomarker for traumatic brain/spinal cord injury (17) and amyotrophic lateral sclerosis (18). Dysfunction of UCHL1 has been proposed to be a contributing factor in Parkinson disease (17). To our knowledge UCHL1 has not been studied in NPC1.

In this study we quantified CSF levels of UCHL1 in cross-sectional and longitudinal CSF samples from a large cohort of individuals with NPC1. Our goal was to investigate whether levels of UCHL1 in CSF correlated with clinical aspects of NPC1 and to determine if this biomarker responded to therapeutic interventions.

2. Results

2.1 Study participant baseline clinical characteristics and demographics

The baseline characteristics for the non-NPC1 comparison group and the NPC1 study cohort are shown in Table 1. The first visit for each patient is considered the baseline. For the NPC1 cohort, the statistical summaries are also stratified by whether the participant was being treated with miglustat at baseline. Of the 94 NPC1 study participants, 50 (53%) of them had one or more follow-up visits. The mean number of visits was 2.4 ± 2 with a median of 2 visits (IQR: 1-3), and the patients were followed for up to 11 years with a median of 0.7 years (IQR: 0-3.7). At baseline, 43 of the 94 NPC individuals (45.7%) were receiving miglustat treatment, and 3 individuals (3.2%) were receiving adrabetadex treatment. Comparison samples were obtained from 35 individuals without NPC1 diagnosis.

Due to inclusion of some older adult NPC1 samples (range 0.3 to 68.1 years), the mean age of the NPC1 cohort was slightly increased at 15.2 ± 14.7 years compared to 9.8 ± 5.8 years (p=0.0369) for the comparison group (range 0.4-21 years). Median ages were 12 and 10.2, respectively. The mean age of NPC1 individuals being treated with miglustat (14.1 ± 11.9) and those not on miglustat at baseline (16.1 ± 16.8, p=0.51) were similar. In the NPC1 cohort, 55/94 (58.5%) were female compared to 10/35 (28.6%) in the comparison group. This difference is primarily due to inclusion of 12 samples from individuals with CTD, an X-linked disorder.

The miglustat untreated and treated cohorts were similar with respect to NPC1 disease severity and burden at baseline (Table 1). Mean age of neurological disease onset was 7.8 ± 10 years with a range of 0.5 to 52 years and median of 5.3 years. The age of neurological onset was comparable between the miglustat treated and untreated groups (p=0.53). The NPC Neurological Severity Score (NPC NSS) is used to characterize the disease progression in NPC. To account for disease heterogeneity, it assesses increasing disease severity in 9 major and 8 minor domains (19). Subsequent work has shown that five of the domains (Ambulation, Fine Motor, Speech, Swallow and Cognition) were highly correlated with the 17-domain NPC NSS and assessed clinically meaningful changes (20, 21). The baseline NPC Neurological Severity Score (NSS) for the entire NPC1 cohort ranged from 0-40 points with a mean of 15.2 ± 10.2. This was comparable between the miglustat treated and untreated groups (p=0.28). The mean 5-domain NPC NSS was 7.9 ±

6.0 points with a median and range of 7.0 and 3.0-23 points, respectively. The 5-domain scores for the untreated and treated miglustat groups were similar (p=0.08). The NPC NSS can be normalized for age. This is referred to as the Annual Severity Increment Score or ASIS (22). The mean ASIS for the NPC cohort was 1.8 ± 2.4 and like both the 17-domain and 5-domain scores, the mean baseline ASIS score was similar irrespective of miglustat therapy (p=0.42).

2.2 Cerebrospinal fluid UCHL1 levels in individuals with NPC1

CSF UCHL1 levels were significantly elevated in the NPC1 cohort relative to the non-NPC1 comparison group. At baseline, the mean UCHL1 level for the NPC1 cohort was 3,491 ± 2,818 pg/mL (median 2,523 pg/ml; range 790 to 16,821 pg/ml) and the mean UCHL1 level for the comparison group was 989 ± 661 pg/ml (median 801 pg/ml; range 363 to 4,066 pg/ml). After log-transforming the UCHL1 values to approximate normality, two-sample t-tests were performed to compare the NPC cohort (n=94) vs the non-NPC controls (n=35). The exponentiated estimated difference between the groups was 3.3 with 95% CI (2.6, 4.1) (FDR-adjusted p<0.0001), meaning the UCHL1 level in the NPC cohort is 3.3-fold higher than in the control group (Figure 1A). We also used a two-sample t-test to compare mean baseline CSF UCHL1 levels in the miglustat untreated NPC1 cohort (n=54) with the non-NPC1 comparison group (n=35) and the exponentiated estimated difference was 3.8 with 95% CI (2.9, 4.9) (FDR-adjusted p<0.0001). Baseline mean CSF UCHL1 levels in the miglustat untreated and treated cohorts were 4,313 ± 3,479 pg/ml and 2,515 ± 1,175 pg/ml, respectively (Figure 1A).

Although the NPC1 phenotype is a continuum, individuals were stratified into age of onset groups as delineated by Vanier (3). Mean CSF UCHL1 levels decreased with increasing age group (Figure 1B, p=0.0088, one-way ANOVA). Age group and (mean CSF UCHL1 levels) were <2 years (4,688 ± 3,844 pg/ml), 2 to < 6 years (3,897 ± 3,521 pg/ml), 6 to <15 years (3,259 ±1,638 pg/ml) and ≥ 15 years (1,899 ± 845 pg/ml).

2.3 Correlation of cerebrospinal fluid UCHL1 levels with NPC1 clinical phenotype and effects of therapeutic interventions

Spearman correlations were determined for CSF UCHL1 levels and measures of NPC1 clinical severity (Table 2a, Figure 2). Consistent with the categorical age stratification, we observed a weak, but statistically significant, negative correlation with between CSF UCHL1 levels and increased age of neurological onset (n=88, r_s=−0.23, FDR-adjusted p=0.0283). Moderate, statistically significant, positive correlations were observed between UCHL1 levels and the ASIS score (n=94, r_s=0.56, FDR-adjusted p<0.0001), the 17-domain NPC-NSS score (n=94, r_s=0.48, FDR-adjusted p<0.0001), and the 5-domain NPC-NSS score (n=94, r_s=0.49, FDR-adjusted p<0.0001).

To explore the potential effect of baseline miglustat therapy we determined Spearman correlations for individuals not being treated with miglustat at baseline (Table 2b) and on miglustat at baseline (Table 2c). Moderate, positive correlations remained significant under both conditions for ASIS, the 5-domain NPC-NSS and the 17-domain NPC-NSS. The weak

negative correlation between CSF UCHL1 levels and ANO observed in the total cohort was not substantiated in the two subgroups.

A linear mixed effect model was used to ascertain the effect of both miglustat and intrathecal 2-hydroxypropyl-β-cyclodextrin (HPβCD, VTS270, adrabetadex) administration on CSF UCHL levels due to the longitudinal nature of the data. Fixed-effect covariates used in the model were age, sex, age of neurological onset, miglustat and intrathecal adrabetadex, and the random effect was individual. Results are presented in Table 3. The introduction of miglustat treatment resulted in a reduction in the UCHL1 biomarker by 30% with 95% CI (17%, 40%) (FDR-adjusted $p<0.001$), on average. The UCHL1 level decreased by 3% in a statistically significant manner with one-year increase in the age of neurological onset. Sex, age, and IT VTS270 did not have statistically significant effects on CSF UCHL1 levels. Spaghetti plots of the longitudinal data for individuals are provided in Supplemental Figure 1.

We previously reported elevated levels of neurofilament light (NEFL) in CSF from individuals with NPC1 (23). The Neuroplex 4 assay used to measure UCHL1 levels in this study, also measured NEFL. Baseline CSF UCHL1 levels correlate significantly ($p<0.0001$) with baseline CSF NEFL levels (Figure 3A). The linear relationship can be modeled by the equation [UCHL1]=2.3*[NEFL]+1133. Interestingly, although the linear relationship remained significant in both cases ($p<0.0001$), the increase in CSF UCHL1 relative to NEFL levels was decreased in individuals being treated with miglustat (Figure 3B). Specifically, the slope of the linear relationship decreased from 3.1 to 1.0.

3. Discussion

Identification and characterization of biomarkers can facilitate both clinical care and therapeutic trials. The potential utility of biomarkers is multifaceted. From a drug discovery perspective, they can provide insight into pathological mechanisms that could be targeted by specific therapeutic agents. In therapeutic trials they have the potential to be used as pharmacodynamic measures or surrogates for a therapeutic response. In the clinic, biomarkers have the potential to be a diagnostic test, provide prognostic guidance, or used to assess disease status.

Several potential biomarkers have been identified in NPC1. Diagnosis of NPC1 has been facilitated by identification of blood-based biomarkers including 3β,5α,6β-cholestane-triol, N-(3β,5α,6β-trihydoxy-cholan-24-oyl)glycine), and N-palmitoyl-O-phosphocholineserine (PPCS, lyso-509) (24). N-(3β,5α,6β-trihydoxy-cholan-24-oyl)glycine or “bile acid B” is stable on newborn blood spot cards and is being investigated for use in newborn screening (25). 24(S)-hydroxycholesterol, an oxysterol produced by neurons in the central nervous system, has proven to be useful as a pharmacodynamic biomarker of improved cholesterol homeostasis in NPC1 (26). Several CSF proteins, in small sample sets, have been shown to be elevated in NPC1 and potentially respond to therapeutic interventions. These include markers of neuronal damage (such as neurofilament light, total tau, fatty acid binding protein 3 and calbindin D (reviewed in (27)) and markers of neuroinflammation (28, 29).

Recent biomarker discovery efforts utilizing either differential proteomics (30) or proximal extension assays (27) have extended the list of potential biomarkers.

To date, the only CSF protein studied in a large cohort of individuals with NPC1 is neurofilament light (NEFL). Agrawal et al. (31) showed that NPC1 CSF NEFL levels were elevated almost 7-fold, positively correlated with increased disease severity, and decreased in individuals treated with miglustat. Dardis et al. (32) showed that NEFL levels are elevated in plasma from individuals with NPC1 and NEFL levels were elevated in samples from individuals with neurological signs/symptoms. These two studies suggest that NEFL levels, a biomarker of neuronal damage, will potentially be useful in monitoring disease progression and therapeutic responses. However, there remains a need to characterize additional biomarkers in large, well phenotyped NPC1 cohorts in order to optimize their use, individually and in combination, as supportive data for clinical efficacy in future therapeutic trials.

This study has several limitations. One limitation is that we used comparison rather than true control samples. Obtaining true control pediatric CSF samples is precluded by both ethical and statutory issues. Thus, in this study we used both residual pediatric clinical samples and CSF samples from two different genetic diseases that are not known to include neurodegeneration. No significant differences were observed between the three types of comparison samples. Another limitation is the use of CSF. While biomarkers in CSF may be more representative of central nervous system pathology, CSF is less easily obtained than blood. Work is in progress to characterize UCHL1 and other potential biomarkers in serum from individuals with NPC1. Although potentially confounded by experimental therapies, it will also be of value to explore the relationship between clinical disease progression and CSF UCHL1 levels in longitudinal samples. Future studies will also be needed to define the kinetics of the reduction of CSF UCHL1 in response to initiation of a therapeutic intervention and a larger cohort may be needed to explore potential therapeutic effect of IT VTS 270.

The goal of this study was to determine the utility of UCHL1 as a biomarker for NPC1. UCHL1 is a relatively brain specific protein which is highly expressed in neurons and considered a biomarker of neuronal cell body damage (17, 33). UCHL1 functions to modulate protein ubiquitination and thus helps regulate the removal of misfolded and nonfunctional proteins. The data in this paper show that CSF UCHL1 levels are elevated over 3-fold and lower levels are associated with less severe (decreased ASIS) and later neurological disease onset. Thus, at a group level, CSF UCHL1 levels potentially have some prognostic utility to stratify individuals by disease severity. We also observed positive correlations between CSF UCHL1 and current disease burden (17- and 5-domain NPC NSS). These data support the conclusion that CSF UCHL1 levels reflect clinically relevant aspects of NPC1.

Although we were not able to demonstrate an effect of intrathecal HPβCD administration, our data show that CSF UCHL1 levels are decreased in individuals treated with miglustat. Miglustat inhibits the synthesis of glycosphingolipids (34). Glycosphingolipids, in addition to unesterified cholesterol, accumulate in NPC1 cells (35, 36). Although miglustat has not

been approved by the FDA for the treatment of NPC1, it has been approved by most other regulatory agencies including the EMA and substantial data exist to support both safety and efficacy (8, 9, 11–14). We have recently published similar data showing that abnormally elevated CSF NEFL levels also decrease in response to miglustat therapy in NPC1 (31). Consistent with this, elevated CSF NEFL and UCHL1 levels show a significant positive correlation with each other. Although both decrease in association with miglustat therapy, the decrease in UCHL1 appears larger. These data strongly support the conclusion that the rate of neuronal damage, and perhaps loss, is slowed by miglustat. Combined with the observation that these biomarkers are correlated with clinical aspects of NPC1, these data also support the use of both UCHL1 and NEFL in future clinical trials as indicators of potential clinical efficacy.

4. Materials and Methods

4.1 Biomaterial collection, clinical phenotyping, and participants

Clinical data and cerebrospinal fluid samples were obtained from individuals with NPC1 enrolled in IRB-approved studies conducted at the NIH Clinical Center. These include a natural history/observational study (NCT00344331), a phase I/II trial of intrathecal HPβCD (NCT01747135) and a phase II/III trial of intrathecal HPβCD (NCT02534844). Either participants or guardians provided written informed consent. Assent was obtained when applicable. Both the 17-domain NPC NSS (19) and the 5-domain NPC NSS (21) were calculated after clinical assessment. Clinical assessments were obtained concordant with sample collection. Annual Severity Increment Score or ASIS was calculated by normalizing the 17-domain NPC NSS for age in years (22). Age of neurological onset and age group stratification were determined by review of clinical history. Cerebrospinal fluid was collected by lumbar puncture, aliquoted, frozen, and stored at −80 °C.

CSF from 94 NPC1 individuals was included in this study. For the non-NPC1 comparison samples we analyzed CSF from 10 pediatric laboratory controls (PLC), 13 samples from individuals with Smith-Lemli-Opitz syndrome (SLOS), and 12 samples from individuals with Creatine Transporter Deficiency (CTD). The anonymized PLC samples were residual CSF collected during a clinically indicated procedure. We had no control over the handling of these samples prior to obtaining them. Both the SLOS (NCT00001721) and CTD (NCT02931682) CSF were obtained as part of IRB approved natural history trials that included written informed consent. Collection, processing, and storage of these samples was the same as for the NPC1 samples.

CSF UCHL1 level were measured using Neuro4-Plex enzyme-linked immunoassay (ELISA) using Simoa™ technology on the Quanterix SR-X platform in a 96-well plate format (Quanterix, Billerica, MA, USA). Briefly, the CSF was diluted according to the manufacturer's protocol with sample diluent and assayed in duplicate. The plates were processed using the 2-step digital immunoassay exactly as outlined in the manufacturer's protocol. All plates had two internal controls in the low (C1) and high (C2) ranges. Samples were run in duplicate. Internal controls were included on all 9 plates and had an inter-assay coefficient of variance (CV%) of 4.6% for the low control (mean 96.3 pg/ml) and 9.1% for the high control (mean 9,525.6 pg/ml).

4.2 Statistical analysis

Data were described using various descriptive statistics as relevant for the variable, such as frequencies and percentages for categorical variables, mean ± standard deviation, median and interquartile range, and range for numerical variables. The distribution of UCHL1 (pg/ml) was assessed for normality and all analyses were performed with log transformed data. Two sample t-tests were performed to compare the NPC group and the non-NPC control group. Spearman correlation coefficients were obtained, with 95% confidence intervals (CIs) and p-values, between UCHL1 and four specific prognostic measurements at baseline.

Longitudinal analyses were carried out via a linear mixed effects model on the log transformed UCHL1 biomarker with miglustat therapy (yes vs no), VTS270 therapy (yes vs no), sex (male vs female), age (years), and age of neurological onset (years) as fixed effect covariates, and subject as a random effect. Exponentiated estimated coefficients, 95% CIs, and p-values describe the effects of the linear mixed effects model.

For the correlation analysis and the linear mixed effects model analysis, where multiple hypothesis tests were conducted to test correlation coefficients against 0 and the regression coefficients against 0, adjusted p-values were obtained using the false discovery rate (FDR) correction (37).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

The data presented in this study are present in this manuscript or available to researchers with ethical approval upon request consistent with National Institutes of Health intramural research policies.

Abbreviations

ANO	Age of Neurological Onset
ASIS	Annual Severity Increment Score
CI	Confidence Interval

CSF	Cerebrospinal Fluid
CTD	Creatine Transporter Deficiency
CV	Coefficient of Variance
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
FDR	False Discovery Rate
HPβCD	2-Hydroxypropyl-β-cyclodextrin (Adrabetadex)
IRB	Institutional Review Board
IT	Intrathecal
NEFL	Neurofilament Light
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NPC	Niemann-Pick Disease, type C
NPC1	Niemann-Pick Disease, type C1
NPC2	Niemann-Pick Disease, type C2
NPC NSS	Niemann-Pick Disease, type C Neurological Severity Score
PLC	Pediatric Laboratory Control
SLOS	Smith-Lemli-Opitz syndrome
UCHL1	Ubiquitin C-terminal hydrolase-L1

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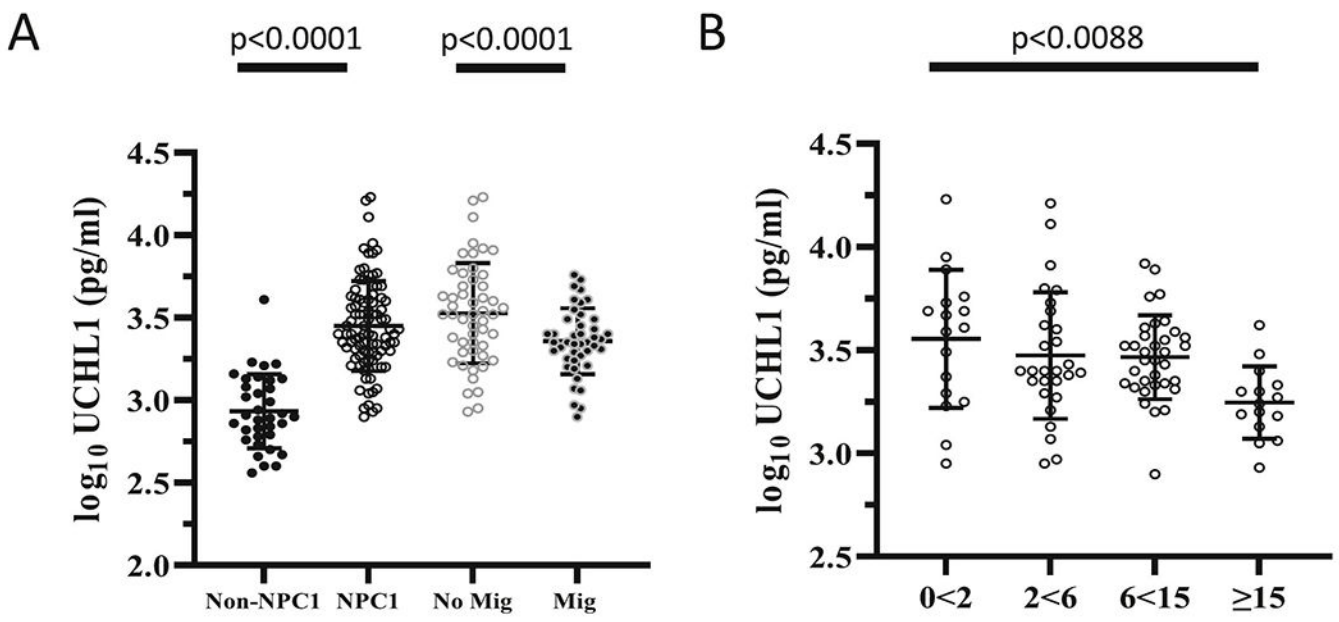

Figure 1. Elevated cerebrospinal fluid UCHL1 levels in individuals with Niemann-Pick Disease, type C1.

CSF UCHL1 levels were $\log_{10}$ transformed to better approximate normality. A) Baseline UCHL1 levels were significantly elevated (adj p<0.0001) in CSF from individuals with NPC1 relative to non-NPC1 comparison samples. Although still elevated, baseline CSF UCHL1 levels were significantly (p<0.0001) lower in the NPC1 samples from individuals being treated with miglustat (Mig). Error bars correspond to mean ± SD, two-sample t-test. **B)** CSF UCHL1 levels were stratified by age groups corresponding to early infantile (0<2 years), late infantile (2<6 years), juvenile (6<15 years), and adolescent/adult (≥ 15 years) disease onset. Mean baseline levels decreased significantly with increased age (p=0.0088, one-way ANOVA). Error bars indicate mean ± SD.

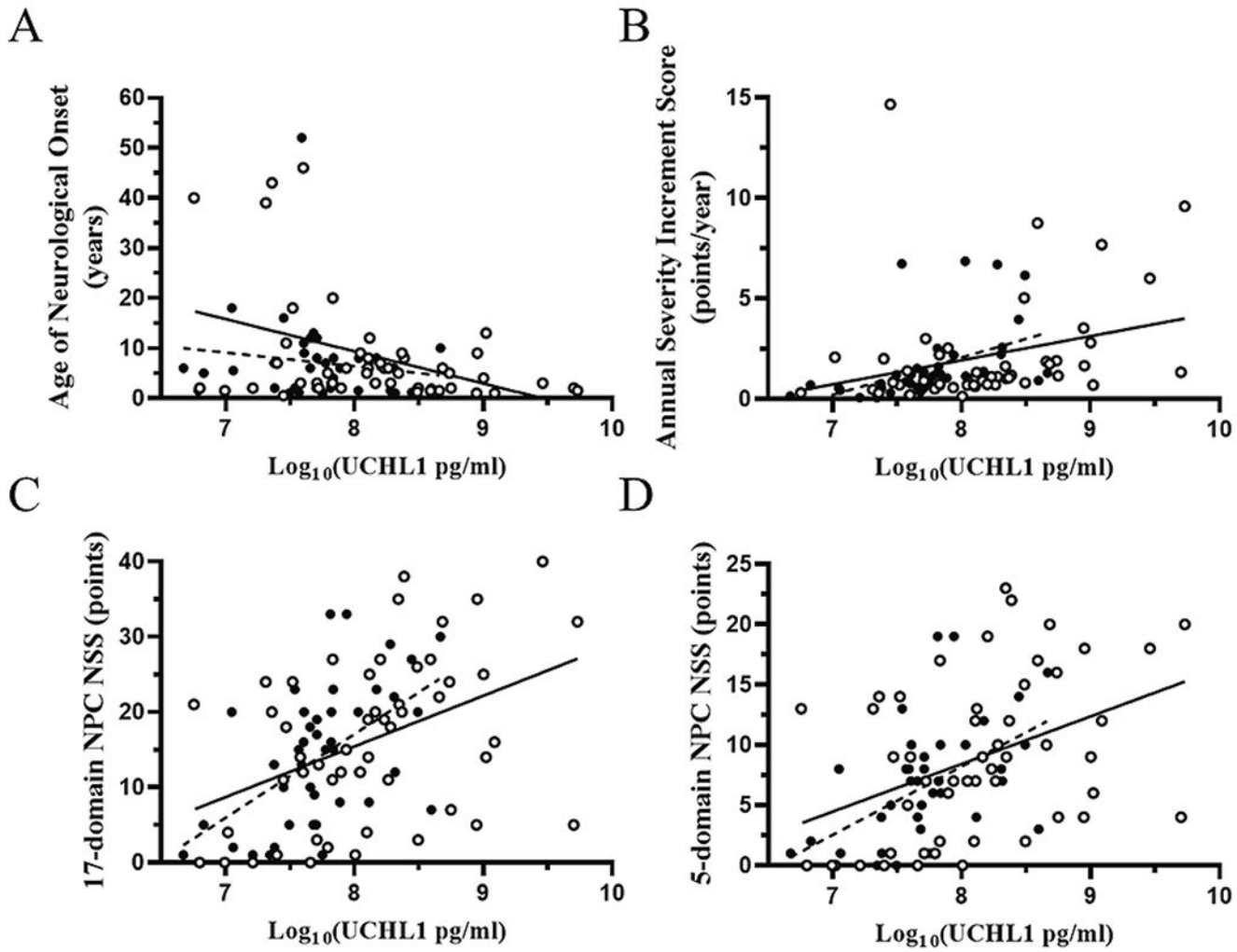

Figure 2. Spearman correlation of cerebrospinal fluid UCHL1 levels with baseline clinical outcome measures.

CSF UCHL1 levels were correlated with **A)** Age of Neurological Onset, **B)** Annual Severity Increment Score or ASIS, **C)** the 17-domain NPC Neurological Severity Score and **D)** the 5-domain NPC Neurological Severity Score (NPC NSS). Open circles and solid line indicate data from untreated individuals. Filled circles and dashed line indicate data from miglustat treated individuals.

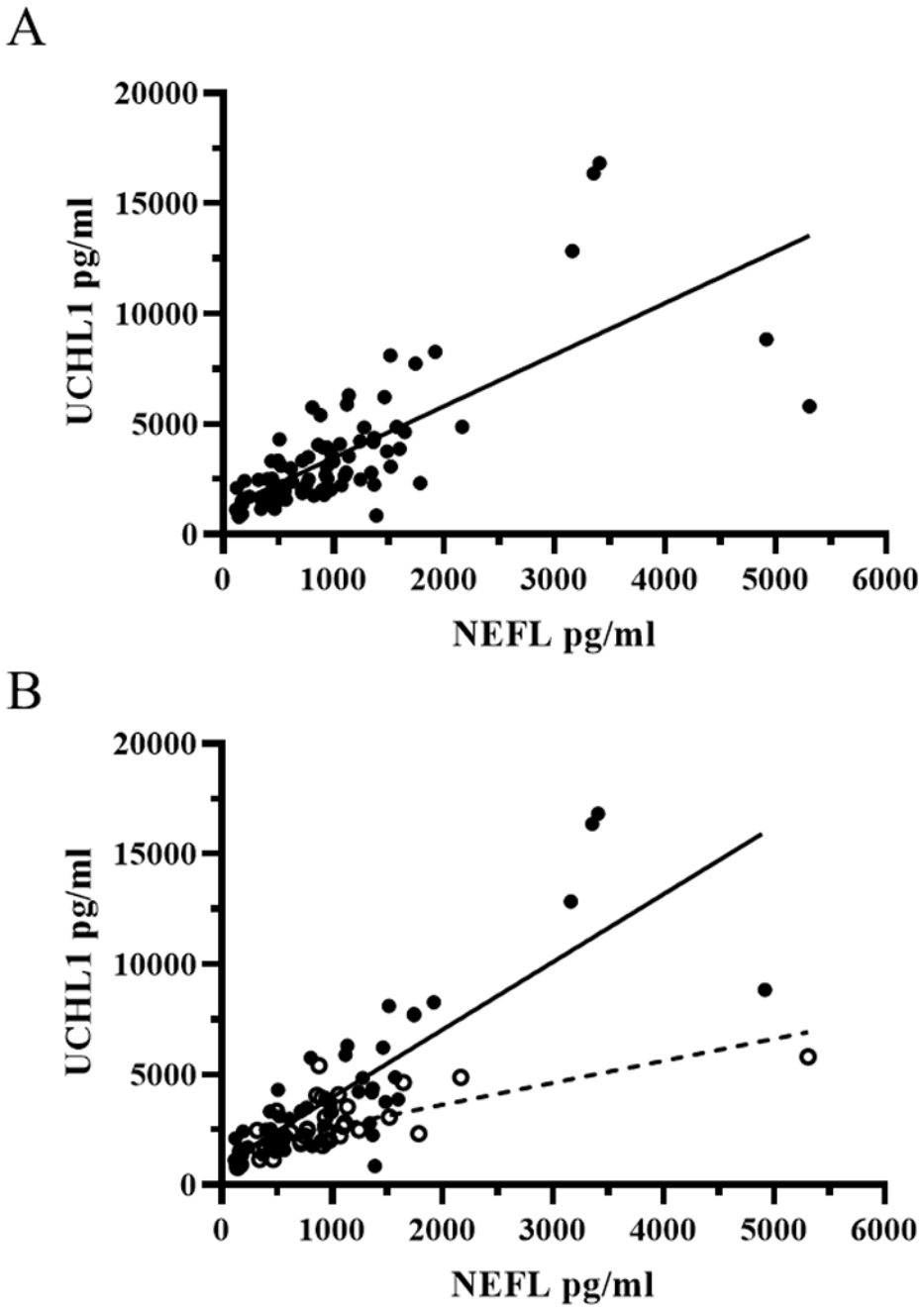

Figure 3. Cerebrospinal fluid levels of UCHL1 and NEFL in individuals with NPC1.

A) There is a significant positive correlation (slope 2.3, $p<0.0001$) between baseline CSF UCHL1 and NEFL levels in individual samples. **B)** Separation of baseline levels by miglustat treatment status. Solid line and filled circles, no miglustat (slope 3.1, $p<0.0001$). Dashed line and open circles, miglustat treated (slope 1.0, $p<0.0001$).

Table 1.

Baseline demographics and clinical phenotype.

Variable	NPC1 (n=94)	Miglustat at Baseline (n=43)	No Miglustat at Baseline (n=51)	Non-NPC1 (n=35)
Age (years)				
Mean ± SD	15.2 ± 14.7	14.1 ± 11.9	16.1 ± 16.8	9.8 ± 5.8
Median (IQR)	12 (4.4 – 20)	12.2 (5.8 – 17)	11.8 (3.8 – 21.3)	10.2 (4.7 – 14.5)
Range	0.3 – 68.1	0.8 – 62.5	0.3 – 68.1	0.4 – 21
Sex (frequency (%))				
Male	39 (41.5%)	20 (46.5%)	19 (37.3%)	25 (71.4%)
Female	55 (58.5%)	23 (53.5%)	32 (62.7%)	10 (28.6%)
ANO (years) *				
Mean ± SD	7.8 ± 10	7.1 ± 8.4	8.4 ± 11.2	
Median (IQR)	5.3 (2 – 8.3)	6 (2 – 8)	5 (2 – 8.5)	N/A
Range	0.5 – 52	1 – 52	0.5 – 46	
17-Domain NPC NSS				
Mean ± SD	15.2 ± 10.2	14 ± 9.2	16.3 ± 11	
Median (IQR)	15 (5.5 – 22)	15 (6 – 20)	16 (6 – 24)	N/A
Range	0 – 40	0 – 33	0 – 40	
ASIS				
Mean ± SD	1.8 ± 2.4	1.6 ± 1.8	2 ± 2.8	
Median (IQR)	1.1 (0.6 – 1.8)	1 (0.6 – 1.4)	(0.7 – 2)	N/A
Range	0 – 14.7	0 – 6.9	0 – 14.7	
5-Domain NPC NSS				
Mean ± SD	7.9 ± 6	6.7 ± 4.9	8.9 ± 6.7	
Median (IQR)	7 (3 – 12)	7 (3 – 9.5)	9 (3 – 13.5)	N/A
Range	0 – 23	0 – 19	0 – 23	
Treatment (frequency (%))				
Miglustat	43 (45.7%)			
IT HPβCD	3 (3.2%)	3 (7%)	0 (0%)	N/A

* n=88. Six individuals were neurologically asymptomatic at baseline. 41 of these individuals were on miglustat and 47 were not on miglustat.

Age of Neurological Onset (ANO); NPC Neurological Severity Score (NPC NSS); Annual Severity Increment Score (ASIS)

Table 2a.

Spearman Correlation between log(UCHL1) and prognostic measures at baseline

Measurement	r_s (95% CI)	FDR-adjusted p-value	Raw p-value
ANO *	−0.23 (−0.42, −0.03)	0.0283	0.0283
17-Domain NPC NSS	0.48 (0.31-0.62)	<0.0001	<0.0001
ASIS	0.56 (0.41-0.69)	<0.0001	<0.0001
5-Domain NPC NSS	0.49 (0.32-0.63)	<0.0001	<0.0001

* n=94 except for ANO where n=88. Six individuals were neurologically asymptomatic at baseline.

Age of Neurological Onset (ANO); NPC Neurological Severity Score (NPC NSS); Annual Severity Increment Score (ASIS)

Table 2b.

Spearman Correlation between log(UCHL1) and prognostic measures at baseline for individuals not receiving treatment at baseline

Measurement*	Spearman Correlation (95% CI)	FDR-adjusted p-value	Raw p-value
ANO	−0.28 (−0.53, 0.003)	0.0530	0.0530
17-Domain NPC NSS	0.46 (0.21, 0.65)	0.0015	0.0007
ASIS	0.53 (0.29, 0.70)	0.0003	<0.0001
5-Domain NPC NSS	0.44 (0.19, 0.64)	0.0015	0.0012

*n=51 except for ANO where n=47. Four individuals not receiving treatment at baseline were neurologically asymptomatic at baseline.

Age of Neurological Onset (ANO); NPC Neurological Severity Score (NPC NSS); Annual Severity Increment Score (ASIS)

Table 2c.

Spearman Correlation between log(UCHL1) and prognostic measures at baseline for individuals treated with miglustat at baseline

Measurement*	Spearman Correlation (95% CI)	FDR-adjusted p-value	Raw p-value
ANO	−0.18 (−0.46, 0.13)	0.2513	0.2513
17-Domain NPC NSS	0.55 (0.29, 0.73)	0.0002	0.0002
ASIS	0.68 (0.48, 0.82)	<0.0001	<0.0001
5-Domain NPC NSS	0.55 (0.30, 0.73)	0.0002	0.0001

*n=43 except for ANO where n=41. Two individuals treated with miglustat at baseline were neurologically asymptomatic at baseline.

Age of Neurological Onset (ANO); NPC Neurological Severity Score (NPC NSS); Annual Severity Increment Score (ASIS)

Table 3.

Longitudinal analysis of CSF UCHL1 levels. Response to therapeutic interventions. Linear mixed-effects model.

Covariate	Effect* (95% CI)	FDR-adjusted p-value	Raw p-value
Miglustat (yes vs. no)	0.70 (0.60, 0.83)	0.0001	<0.0001
ANO (years)	0.97 (0.95, 0.99)	0.0344	0.0172
IT HPβCD (yes vs. no)	1.17 (0.98, 1.40)	0.1366	0.0911
Sex (male)	0.83 (0.65, 1.06)	0.1538	0.1282
Age (years)	1.01 (0.99, 1.02)	0.4282	0.4282

Total of 217 CSF samples from 88 NPC1 participants.

*Effect% represents the percentage change in the UCHL1 level with one unit increase in the covariate.

Age of Neurological Onset (ANO), Intrathecal (IT), 2-hydroxypropyl-β-cyclodextrin (HPβCD).