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Elevated Cerebrospinal Fluid Total Tau in Niemann-Pick Disease Type C1: Correlation With Clinical Severity and Response to Therapeutic Interventions

Niamh X. Cawley[1] | Ruyu Zhou[2] | Nicole M. Farhat[1] | James Iben[3] | Derek M. Alexander[1] | Rachel A. Luke[1] | Cameron J. Padilla[1] | Hibaaq O. Mohamed[1] | Orsolya K. Albert[4] | Kendall P. Robbins[4] | Samar Rahhal[1] | An Dang Do[5] | Elizabeth Berry-Kravis[4] | Stephanie M. Cologna[6] | Fang Liu[2] | Forbes D. Porter[1]

[1]Section on Molecular Dysmorphology, Division of Translational Medicine, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA | [2]Applied and Computational Mathematics and Statistics, University of Notre Dame, Indiana, USA | [3]Molecular Genomics Core, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA | [4]Departments of Pediatrics, Neurological Sciences, and Anatomy and Cell Biology, Rush University Medical Center, Chicago, Illinois, USA | [5]Unit on Cellular Stress in Development and Diseases, Division of Translational Medicine, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA | [6]Department of Chemistry and Laboratory of Integrative Neuroscience, University of Illinois Chicago, Chicago, Illinois, USA

Correspondence: Forbes D. Porter (fdporter@mail.nih.gov)

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ABSTRACT

Niemann-Pick disease, type C1 (NPC1) is an inborn error of intracellular cholesterol transport. Impaired function of NPC1 leads to endolysosomal accumulation of unesterified cholesterol, which results in progressive neurodegeneration. Although the age of onset is variable, classical NPC1 is a pediatric disease. Identification of biomarkers that correlate with clinical phenotype and respond to therapeutic interventions will be essential for developing effective therapeutic interventions. Aβ peptides and Tau protein are primary components of amyloid plaques and neurofibrillary tangles, respectively, which are major pathological features in neurodegenerative disorders. Cerebrospinal fluid (CSF) levels of total Tau, a biomarker of axonal damage, were elevated ~3-fold ($p < 0.0001$) in 106 individuals with Niemann-Pick disease, type C1, relative to age-appropriate comparison samples. Baseline CSF total Tau levels correlated with clinical measures of disease severity. Specifically, CSF total Tau levels decreased with increased age of neurological onset ($r_s = -0.42$, FDR adj. $p < 0.0001$) and increased with increased Annual Severity Increment Score ($r_s = 0.52$, FDR adj. $p < 0.0001$). Baseline CSF total Tau levels were decreased 40% ($p = 0.0066$) in individuals being treated with miglustat, and longitudinal analysis substantiated this observation with a 40% decrease ($p < 0.0001$, 95% CI 32%–47.4%). Longitudinal analysis also showed a significant ($p = 0.004$) decrease of 19% (95% CI 7%–30%) in total Tau levels associated with intrathecal 2-hydroxypropyl-β-cyclodextrin therapy.

Abbreviations: 2HPβCD, 2-hydroxypropyl-β-cyclodextrin (VTS270 Adrabetadex); ANO, age of neurological onset; ASIS, Annualized Severity Index Score; CI, confidence interval; CSF, cerebrospinal fluid; CV, coefficient of variance; FDA, Food and Drug Administration; FDR, false discovery rate; IRB, Institutional Review Board; IT, Intrathecal; NPC, Niemann-Pick disease, type C; NSS, Neurological Severity Score.

Niamh X. Cawley and Ruyu Zhou contributed equally.

These data show that CSF total Tau levels are significantly increased in individuals with NPC1, positively correlated with increased disease severity, and respond to therapeutic interventions.

1 | Introduction

Niemann-Pick disease, type C1 (NPC1) is an ultrarare, autosomal recessive disorder with an estimated incidence of ~1/100,000 [1, 2]. NPC1 is a lysosomal disease caused by pathological variants of *NPC1*. The NPC1 protein functions to transport cholesterol out of the endolysosomal compartment and make it bioavailable for cellular function. Impaired NPC1 function results in the endolysosomal storage of unesterified cholesterol and other lipids, and paradoxically a functional cholesterol deficiency. Although heterogeneous with respect to age of onset and specific sign/symptom complex in individuals with NPC1, the phenotype is predominantly characterized by progressive cerebellar ataxia and cognitive impairment.

Therapeutic trials for NPC1 are difficult due to both the rarity and clinical heterogeneity of the disease. These two factors are compounded by the insidious and slow progression of neurological signs and symptoms. NPC1 neuropathology progresses over years. Until recently, there were no disease-modifying USA Food and Drug Administration (FDA) approved therapies for NPC1. The FDA has now approved arimoclomol, a protein-stabilizing drug (Myplyffa, Zevra Therapeutics) in combination with miglustat, a glycosphingolipid inhibitor labeled for the treatment of Gaucher disease, as the first US approved treatment for NPC1. Miglustat has routinely been used off-label and shown to have real-world clinical efficacy for individuals with NPC1 [3, 4]. In addition, the FDA recently approved the first stand-alone treatment for NPC1, N-acetyl-L-leucine (AQNEURSA, IntraBio Inc).

Biomarkers that reflect disease neuropathology and respond to therapeutic interventions may be useful in supporting the efficacy of experimental therapeutic interventions. In a promising move for rare, slowly progressive neurodegenerative diseases where classical placebo-controlled trials are not feasible, the USA Federal Drug Administration approved tofersen for a rare form of amyotrophic lateral sclerosis based on a reduction of plasma neurofilament light chain levels [5].

Cerebrospinal fluid (CSF) protein biomarkers have been studied in common neurodegenerative disorders such as Alzheimer disease and tauopathies [6]. In particular, these studies have identified abnormal levels of Tau. Tau is a microtubule-associated protein present in neuronal axons. Tau is encoded by *MAPT*. Increased CSF total Tau levels are indicative of neuronal/axonal damage. Hyperphosphorylation of Tau contributes to the formation of neurofibrillary tangles, a neuropathological finding in Alzheimer disease as well as other tauopathies such as progressive supranuclear palsy and frontotemporal dementia [7]. Tau has been previously implicated in NPC1 pathology. Hyperphosphorylated Tau, the precursor to neurofibrillary tangles, has been reported in *Npc1*$^{-/-}$ mouse brain tissue [8]. Both *Mapt*$^{+/-}$:*Npc1*$^{-/-}$ and *Mapt*$^{-/-}$:*Npc1*$^{-/-}$ mice manifest a more severe phenotype with decreased survival than *Mapt*$^{+/+}$:*Npc1*$^{-/-}$ mice, perhaps due to decreased autophagy [9, 10]. Hyperphosphorylated Tau and neurofibrillary tangles are observed in postmortem NPC1 brain tissue [11–14]. In a small study, we previously reported elevated total Tau, but not phosphorylated Tau, in CSF from individuals with NPC1 [15, 16]. These prior data suggested that CSF total Tau levels decreased in response to the initiation of miglustat therapy. Increased CSF total Tau levels likely reflect ongoing neurodegeneration. More recently, Gonzalez-Ortiz et al. reported elevated levels of pTau(217) and pTau(231) in the plasma of NPC1 individuals that correlated with the age of disease onset and Annual Severity Increment Score, suggesting these phosphorylated Tau forms may represent a blood-based biomarker for NPC1 [17].

In this study, we sought to expand upon our initial reports by quantifying total Tau in cross-sectional and longitudinal CSF samples from a large cohort of individuals with NPC1. Our goal was to determine if CSF levels of total Tau correlated with clinical aspects of the disease and to determine if CSF total Tau levels responded to therapeutic interventions.

2 | Results

2.1 | Study Participant Baseline Clinical Characteristics and Demographics

Table 1 provides the baseline, first visit, demographic, and clinical characteristics for the non-NPC1 comparison group and the NPC1 cohort. Baseline for a subject is defined as the first visit for the subject with an available CSF total Tau measurement. The NPC1 cohort is also stratified by baseline miglustat therapy. Baseline CSF total Tau levels were quantified in 106 individuals with NPC1 and in 22 non-NPC1 samples. The mean age of the NPC1 cohort, 14.7 ± 14.1 years, was slightly older than the mean age of the non-NPC1 comparison group (11.3 ± 6.1 years, $p = 0.27$, unpaired *t*-test). This is likely due to the inclusion of some adult NPC1 cases. Median age, 11.8 and 11.6 years respectively, was similar. With respect to sex, 53% of the NPC1 cohort were female compared to 41% in the comparison group. In the NPC1 cohort, age of neurological onset (ANO) and Annual Severity Increment Score (ASIS), clinical parameters of disease severity, were 7.7 ± 9.5 years and 1.9 ± 2.4 points/year, respectively. The NPC Neurological Severity Score (NSS) is an indicator of current disease burden. The full 17-domain [18] and 5-domain subscale [19] mean values at baseline were 15.4 ± 10.5 and 8.2 ± 6.0 points, respectively. At baseline, 47/106 (44.3%) of the individuals with NPC1 were receiving miglustat treatment. The miglustat untreated and treated cohorts were similar with respect to clinical phenotype at baseline (Table 1). There were no significant differences between the miglustat untreated and treated cohorts for age ($p = 0.91$), ANO ($p = 0.88$), ASIS ($p = 0.22$), 17-domain NPC NSS ($p = 0.77$) or 5-domain NPC NSS ($p = 0.24$). At baseline, no participants were receiving arimoclomol, but 5 individuals (4.7%) were receiving intrathecal 2-hydroxypropyl-β-cyclodextrin (VTS270, adrabetadex) treatment.

TABLE 1 | Baseline demographics and clinical characteristics.

Variable	Non-NPC1 ($n=22$)	NPC1 ($n=106$)	No miglustat ($n=59$)	Miglustat ($n=47$)
Age (years)				
Mean ± SD	11.3 ± 6.1	14.7 ± 14.1	14.9 ± 15.8	14.6 ± 11.8
Median (IQR)	11.6 (5.6–16.9)	11.8 (4.4–19.3)	10.0 (3.5–20.4)	12.4 (6.8–17.4)
Range	1.4–21	0.3–68.1	0.25–68.1	0.8–62.5
Sex				
Male	13 (59.1%)	50 (47.2%)	25 (42.4%)	25 (53.2%)
Female	9 (40.9%)	56 (52.8%)	34 (57.6%)	22 (46.8%)
Treatment (%)	N/A			
Miglustat		47 (44.3%)	—	—
IT HPβCD		5 (4.7%)	2 (3.4%)	3 (6.4%)
Arimoclomol		0 (0%)	0 (0%)	0 (0%)
17-domain NPC NSS	N/A			
Mean ± SD		15.4 ± 10.5	15.6 ± 10.8	15.0 ± 10.1
Median (IQR)		15.0 (6.3–21.0)	16.0 (5.5–21.5)	15.0 (7.5–20.0)
Range		0–42	0–40	0–42
5-domain NPC NSS	N/A	8.2 ± 6.0	8.8 ± 6.5	7.4 ± 5.4
Mean ± SD		7.5 (3.3–12)	9 (3–13.5)	7 (3.5–10)
Median (IQR)		0–23	0–23	0–22
Range				
ANO (years)[a]	N/A			
Mean ± SD		7.7 ± 9.5	7.9 ± 10.3	7.6 ± 8.6
Median (IQR)		6.0 (2–9)	5 (2–8.3)	6 (2–9)
Range		0.5–52	0.5–46	1–52
ASIS (points/year)[b]	N/A			
Mean ± SD		1.9 ± 2.4	2.2 ± 2.8	1.6 ± 1.9
Median (IQR)		1.1 (0.7–1.9)	1.1 (0.7–2.0) 0.13–14.7	1.0 (0.6–1.5)
Range		0.06–14.7	0.13–14.7	0.06–6.9

Abbreviations: ANO, age of neurological onset; ASIS, Annual Severity Increment Score; HPβCD, 2-hydroxypropyl-β-cyclodextrin (VTS270, adrabetadex); IT, intrathecal; N/A, not applicable; NSS, Neurological Severity Score.
[a] $n=101$ for the NPC1 cohort, $n=45$ for miglustat at baseline, and $n=56$ for no miglustat at baseline.
[b] $n=101$ for NPC1 cohort, $n=46$ for miglustat at baseline, and $n=55$ for no miglustat at baseline.

2.2 | Cerebrospinal Fluid Levels of Total Tau Are Increased in Individuals With Niemann-Pick Disease, Type C1

In concordance with earlier studies [15, 16], we observed increased levels of total Tau in CSF from individuals with NPC1 relative to non-NPC1 comparison samples (Figure 1A). At baseline, mean total Tau levels for the NPC1 cohort were 422.2 ± 391.2 pg/mL, while for the non-NPC1 comparison samples, the mean was 121.9 ± 64.9 pg/mL. After log transformation to approximate normality, two-sample *t*-tests were performed to compare the NPC cohort ($n=106$) vs. the non-NPC comparison samples ($n=22$) on CSF total Tau levels (Figure 1A). The exponentiated estimated difference between the groups was 2.95 with a 95% CI (2.26, 3.86) (FDR-adjusted $p<0.0001$), meaning the CSF total Tau level in the NPC cohort is 2.95-fold of that in the control group.

Although the NPC1 phenotype is a continuum, individuals were stratified into age of neurological onset groups as delineated by Vanier [1]. Age group mean total Tau levels were < 2 years (507.7 ± 470.3 pg/mL), 2 to < 6 years (568.7 ± 518.1 pg/mL), 6 to < 15 years (359.1 ± 218.2 pg/mL) and ≥ 15 years (135.0 ± 38.1 pg/mL). Log transformed data are plotted in Figure 1B. Median

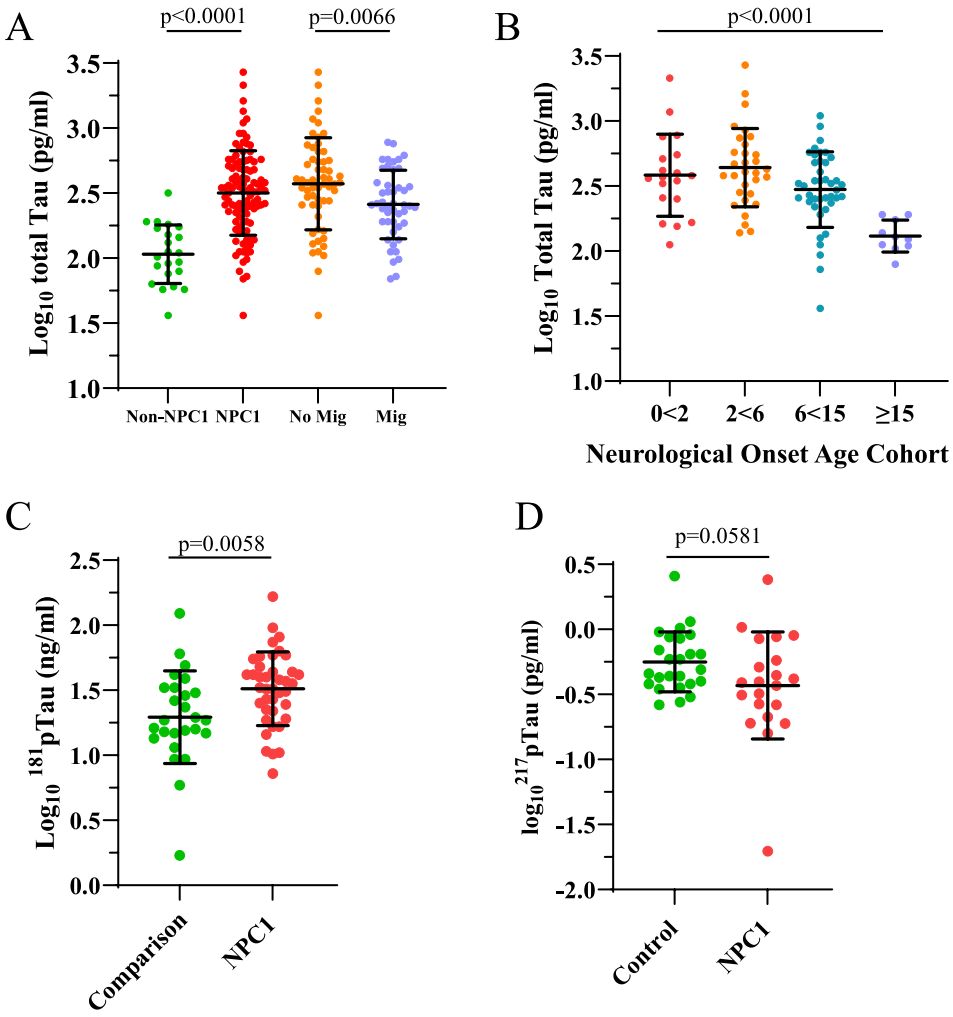

FIGURE 1 | Baseline CSF total Tau levels. (A) Log_{10} CSF total Tau levels were elevated ($p < 0.0001$, Mann–Whitney-*U* test) in individuals with NPC1 relative to age-appropriate non-NPC1 comparison samples. Baseline CSF total Tau levels were significantly ($p = 0.0066$, Mann–Whitney-*U* test) lower in individuals with NPC1 being treated with miglustat (Mig). (B) Log_{10} CSF total Tau levels decreased ($p < 0.0001$, Kruskal–Wallis) toward normal when categorized by age of neurological onset category. (C) CSF log_{10} P-Tau(181) (ng/mL) in NPC1 and age-appropriate comparison samples. (D) Plasma log_{10} pTau(217) levels in control and NPC1 samples.

CSF total Tau levels differed across the age groups ($p < 0.0001$, Kruskal–Wallis test), with a decreasing trend over the age groups < 6 years old, 6 to < 15 years old, and > 15 years old ($p < 0.0001$, Kruskal–Wallis test).

2.3 | MAPT Polymorphisms

MAPT, the gene encoding Tau, is present in two major haplotypes, H1 and H2. The H1 haplotype has been identified as a risk factor for two tauopathies, corticobasal degeneration and progressive supranuclear palsy, and the H1 haplotype is associated with increased Tau expression [7]. *MAPT* haplotype information was available for 74 of the NPC1 individuals in this study; thus, we examined the relationship between *MAPT* haplotype and baseline CSF total Tau levels. No difference was observed ($p = 0.94$, one-way ANOVA, Figure S1). Mean CSF total Tau levels for H1H1, H1H2, and H2H2 individuals were 390.4 ± 283.6, 391.5 ± 230.5, and 349.3 ± 133.5 pg/mL, respectively.

2.4 | Phosphorylated Tau

Hyperphosphorylation of Tau predisposes one to the formation of neurofibrillary tangles, a pathological hallmark in Alzheimer disease and tauopathies. Phosphorylation of threonine 181 is characteristic of Alzheimer disease. We previously did not observe increased threonine 181 phosphorylated Tau (pTau(181)) in CSF from individuals with NPC1 [16]. In this study, mean values for the comparison group and NPC1 cohort were 25.60 ± 26.54 and 39.83 ± 28.40 ng/mL, respectively. After log transformation to approximate normality, this relatively minor increase appeared significant (Figure 1C, $p = 0.0074$, Mann–Whitney-*U* test). Recently, pTau(217) has been reported to be an accurate blood-based biomarker for Alzheimer disease [20] and a potential biomarker for NPC1 [17]. Given the pathological overlap with some aspects of NPC1 pathology, we measured plasma pTau(217) levels in a cohort of individuals with NPC1. In contrast to elevated levels of plasma pTau(217) found in Alzheimer disease and that found in NPC1 individuals reported by Gonzalez-Ortiz et al. [17],

we found the mean plasma pTau(217) level was not significantly different in individuals with NPC1 (0.53 ± 0.51 pg/mL) relative to control values (0.66 ± 0.47 pg/mL). Log transformed values are shown in Figure 1D ($p = 0.064$, Mann–Whitney-*U* test). Given these results and the significant overlap, neither CSF pTau(181) nor plasma pTau(217) are likely to be a useful biomarker for NPC1, although the reason for the difference between our results here and those reported by Gonzalez-Ortiz et al. will need to be studied further.

2.5 | Baseline Cerebrospinal Fluid Levels of Total Tau Are Decreased in Individuals Treated With Miglustat

Baseline CSF total Tau levels were compared in NPC1 individuals being treated with miglustat versus individuals not on miglustat. The mean total Tau in the miglustat untreated and treated cohorts was 514.5 ± 481.8 and 306.4 ± 178.8 pg/mL, respectively. Log transformed data is shown in Figure 1A. Baseline CSF total Tau levels were significantly decreased in the miglustat treated cohort ($p = 0.0066$, Mann–Whitney-*U* test) relative to the miglustat untreated NPC1 individuals.

2.6 | Correlation of Baseline Cerebrospinal Fluid Total Tau Levels With NPC1 Clinical Phenotype

Baseline CSF total Tau levels were correlated with age of neurological onset (ANO), Annualized Severity Index Score (ASIS), the 17-domain NPC Neurological Severity Score (NPC NSS) and the 5-domain NPC NSS. Spearman correlations are shown in Table 2 and data are plotted in Figure 2.

A significant moderate negative ($n = 101$, $r_s = -0.42$, FDR-adjusted $p < 0.0001$) association was observed between CSF total Tau levels and ANO. A significant moderate positive correlation ($n = 101$, $r_s = 0.52$, FDR adjusted $p < 0.0001$) was observed between CSF total Tau levels and ASIS. Both ANO and ASIS provide an assessment of disease severity [21]. Specifically, earlier ANO and higher ASIS values are consistent with increased disease severity. Total Tau levels did not correlate with either the 17-domain NPC NSS ($p = 0.42$) or the 5-domain NPC NSS ($p = 0.42$). The NPC NSS provides an assessment of current disease burden [18, 19].

TABLE 2 | Association of CSF total Tau and clinical outcome measures.

Measurement	r_s (95% CI)	FDR-adjusted *p*	Raw *p*
ANO ($n = 101$)	−0.42 (−0.57, −0.25)	< 0.0001	< 0.0001
ASIS ($n = 101$)	0.52 (0.36, 0.65)	< 0.0001	< 0.0001
5-domain NPC NSS ($n = 106$)	0.09 (−0.10, 0.28)	0.418	0.340
17-domain NPC NSS ($n = 106$)	0.08 (−0.11, 0.27)	0.418	0.418

Abbreviations: ANO, age of neurological onset; ASIS, Annualized Severity Index Score; CI, confidence interval; FDR, false discovery rate; NSS, Neurological Severity Score.

We explored the effect of miglustat on these baseline correlations (Figure S2). Moderate negative Spearman correlations were observed for ANO in both miglustat untreated ($r_s = -0.46$, $p = 0.0004$) and miglustat treated ($r_s = -0.38$, $p = 0.0098$). Similarly, significant positive Spearman correlations remained for ASIS values corresponding to both the miglustat untreated ($r_s = 0.41$, $p = 0.0020$) and miglustat treated ($r_s = 0.66$, $p < 0.0001$) cohorts. These values were qualitatively similar to the correlations observed with the entire NPC1 cohort.

2.7 | Therapeutic Efficacy of Miglustat and Intrathecal 2-Hydroxypropyl-β-Cyclodextrin

Longitudinal data on CSF total Tau levels were used to ascertain the therapeutic potential of both miglustat and intrathecal 2-hydroxypropyl-β-cyclodextrin (2HPβCD, VTS270, adrabetadex). To account for the longitudinal nature of the data, we used a linear mixed-effect model. The data set consisted of 262 measurements from 101 individuals with NPC1. Fixed-effect covariates used in the model were baseline age, the time elapsed since the baseline visit, sex, age of neurological onset, miglustat and intrathecal 2HPβCD, and the random effects were individual and the time elapsed since the baseline visit. Results are presented in Table 3 and Figure 3. We did not observe a statistically significant effect on CSF total Tau levels with either age or sex. Miglustat therapy was associated with a reduction in CSF total Tau levels of 40% (FDR-adjusted $p < 0.0001$). Intrathecal 2HPβCD therapy was associated with a reduction in CSF total Tau levels of 19% (FDR-adjusted $p = 0.008$). These data support the conclusion that both miglustat and intrathecal 2HPβCD decrease neurological damage in individuals with NPC1.

3 | Discussion

Biomarkers can provide insight into both pathological mechanisms and the effectiveness of therapeutic interventions to impact disease pathology. Tau protein is involved in the formation of neurofibrillary tangles, a pathological finding that has been reported in brain tissue from individuals with NPC1 [11–14]. In addition, Villemagne et al. [22] have demonstrated Tau protein deposition in NPC1 brains utilizing ^{18}F-AV1451 PET scanning. We previously reported increased levels of CSF total Tau in a smaller cohort of individuals, and our data suggested that levels decreased with miglustat therapy [15, 16]. The goal of this study was to confirm and extend those initial observations in a larger cohort. Specifically, we wanted to examine the relationships between CSF total Tau and both phenotypic measures of disease severity/burden and the response of CSF total Tau levels to therapeutic interventions.

In this study, we confirmed that CSF total Tau levels are markedly elevated in NPC1, and we extended this observation to show that increased CSF total Tau levels are indicative of increased disease severity. Specifically, increased CSF total Tau levels are associated with a younger age of neurological onset

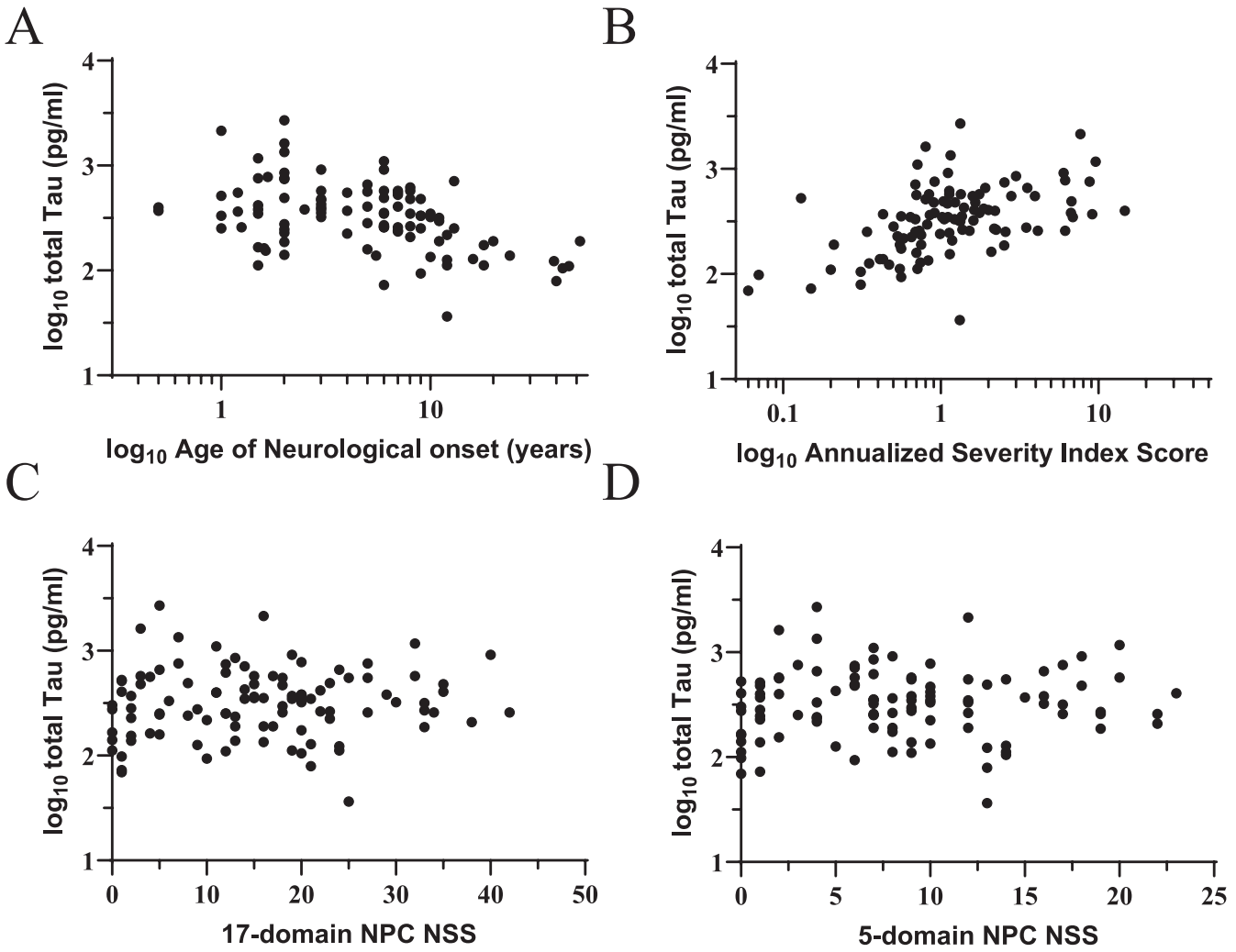

FIGURE 2 | Spearman correlations between CSF total Tau levels and measures of clinical severity and disease burden in individuals with NPC1. Spearman correlations were determined for Age of Neurological Onset (A), Annualized Severity Index Score (B), 17-domain NPC Neurological Severity Score (C), and 5-domain NPC Neurological Severity Score (D). A significant negative association was observed for $\log_{10}$ CSF total Tau levels and Age of Neurological onset. A significant positive association was observed for $\log_{10}$ CSF total Tau and the Annualized Severity Index Score. Neither the concurrent 17- nor 5-domain NPC Neurological Severity Scores were correlated with CSF total Tau levels.

and a higher Annual Severity Increment Scores. Notably, we confirmed that treatment with miglustat significantly reduces CSF total Tau levels in individuals with NPC1. At baseline, CSF total Tau levels were lower in NPC1 individuals on miglustat compared to those not on miglustat, and analysis of our longitudinal data showed a 40% reduction in CSF total Tau levels in samples from individuals treated with miglustat. Miglustat therapy has been shown, in multiple studies, to slow disease progression in NPC1 [3, 4, 23]. We have also shown that other CSF biomarkers of neuronal damage, neurofilament light [24] and UCHL1 [25] also decrease in NPC1 individuals treated with miglustat. Along with the current data on total Tau, these data suggest that CSF total Tau levels in response to miglustat therapy reflect a decrease in the progression of disease and strongly support the case for the clinical efficacy of miglustat. Although our data do not contain samples from individuals treated with arimoclomol, it would be interesting to measure CSF total Tau from NPC1 individuals receiving arimoclomol plus miglustat in the future. Similarly, obtaining biomarker data on individuals being treated with NALL may help determine if it has disease-modifying activity. Due to the multi-syndromic nature of NPC1, we believe that a multi-treatment approach should be beneficial to NPC1 individuals.

While analysis of CSF biomarkers may directly inform on the neurological status of NPC1 disease, obtaining biomarkers from the blood would be optimal. Recently, neurofilament light protein (NfL) has been shown to be detectable in serum/plasma and correlates with NPC1 neurological disease [17, 26, 27]. In addition, pTau(217) has emerged as a strong blood-based candidate as a biomarker for Alzheimer disease. In addition, while this paper was under review, Gonzalez-Ortiz et al. [17] reported elevated levels of pTau(217) in individuals with NPC1. This is not consistent with our results for pTau(217) which showed no difference between NPC and control samples. The reason for this discrepancy is not readily apparent given that the same assay was used. Further studies with age-appropriate controls and accounting for disease and treatment status will be required to discern the reason for this difference.

TABLE 3 | Longitudinal analysis of CSF total Tau and therapeutic interventions.

Covariate	Exp (estimated coefficient) (95% CI)[a]	FDR-adjusted *p*	Raw *p*
Baseline age (years)	0.984 (0.966, 1.002)	0.147	0.084
Time since baseline (years)	0.993 (0.951, 1.038)	0.766	0.766
Sex (male)	0.831 (0.648, 1.066)	0.201	0.144
ANO (years)	0.987 (0.959, 1.014)	0.395	0.339
Miglustat (yes)	0.600 (0.526, 0.685)	< 0.0001	< 0.0001
IT 2HPβCD (yes)	0.808 (0.701, 0.931)	0.008	0.004

Abbreviations: 2HPβCD, 2-hydroxypropyl-β-cyclodextrin; ANO, age of neurological onset; CI, confidence interval; FDR, false discovery rate; IT, intrathecal.

[a](exp(estimated coefficient)−1)×100% represents the percentage change in CSF total TAU level with one unit increase in a numerical covariate (baseline age, time and ANO); for the categorical covariates sex, miglustat, and IT 2HPβCD, it represents the percentage change in CSF total TAU level between male vs. female for sex and yes vs. no for miglustat, and IT 2HPβCD, respectively.

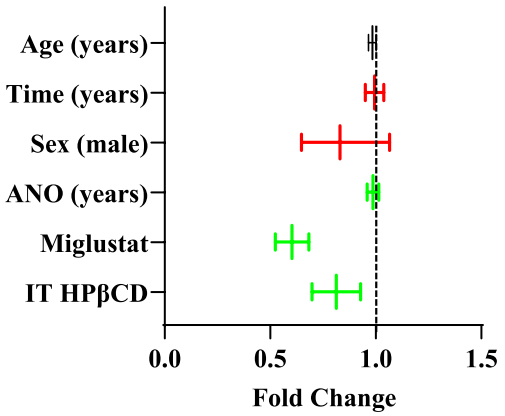

FIGURE 3 | Longitudinal analysis of CSF total Tau. Forest plot of fold change in total Tau levels with one unit increase in a covariate and 95% confidence intervals for the six covariates evaluated using a linear mixed effects model. Significant decreased total Tau levels were associated with both miglustat and intrathecal 2HPβCD therapy.

We also observed a 19% reduction in CSF total Tau levels in individuals treated with intrathecal 2HPβCD. Preclinical studies in both NPC1 mice [28, 29] and cats [30] strongly support the efficacy of 2HPβCD both in terms of decreased neuropathology and increased survival. In addition, a phase 1/2 study of intrathecal 2HPβCD demonstrated a positive pharmacodynamic effect, a decrease in CSF biomarkers of neuronal damage, and a decrease in the expected rate of disease progression over 18 months [31]. The CSF total Tau data we are now reporting are consistent with the prior biomarker data showing decreased neuronal damage and support the potential clinical efficacy of intrathecal 2HPβCD.

This study has several limitations. One potential critique is the lack of true control CSF samples. This limitation is inherent to a pediatric study, as the collection of control CSF is precluded by ethical and regulatory constraints. To minimize the potential impact of this issue and to control for variation in collection/processing, we used both residual pediatric laboratory samples and CSF obtained from individuals with SLOS that were collected and processed like the NPC1 samples. SLOS is a neurodevelopmental disorder, but unlike NPC1, does not include neurodegeneration. No difference was noted in analyte levels between these two comparison groups. This study does not address the kinetics of the reduction of CSF total Tau levels in response to the initiation of either miglustat or IT 2HPβCD therapy. This will be an important parameter to determine the full usefulness of CSF total Tau in therapeutic trials.

4 | Conclusions

The data presented in this paper conclusively show that CSF total Tau levels are significantly increased in individuals with NPC1. Consistent with the concept that increased CSF total Tau levels reflect increased axonal damage, we found that increased total Tau levels were associated with a more severe NPC1 phenotype. Furthermore, we demonstrate that miglustat therapy, a drug shown to have clinical efficacy in slowing NPC1 disease progression, is associated with decreased CSF levels of total Tau levels. Our current data also support the potential therapeutic efficacy of IT 2HPβCD therapy. CSF total Tau, potentially in combination with other biomarkers of NPC1 neuropathology, may have utility with respect to assessing disease severity and supporting therapeutic efficacy.

5 | Materials and Methods

5.1 | Participants and Study Approval

Individuals with NPC1 were enrolled in clinical protocols conducted at the NIH Clinical Center or Rush University Medical Center (RUMC). These protocols included a natural history/observational study (NCT00344331), phase I/II and phase II/III studies of intrathecal VTS-270/adrabetadex (NCT01747135 and NCT02534844), and an expanded access program for access to intrathecal VTS-270/adrabetadex for patients with NPC1 who were unable to enroll in trials (IND 119856). These clinical protocols were initially approved by the NICHD IRB, with ongoing approval provided by the NIH Clinical Center IRB. The RUMC expanded access protocol and the phase II/III trial were approved by the RUMC IRB. Written informed consents were obtained from either participants or guardians. Procedures and risks were described to participants at an age/cognitively appropriate level, and assent was obtained when applicable.

The non-NPC1 comparison group consisted of nine anonymized residual CSF samples from pediatric patients (Pediatric Laboratory Controls, PLC) who underwent CSF collection for a clinical indication and 13 CSF samples collected from individuals with Smith–Lemli–Opitz syndrome (SLOS). SLOS is a malformation syndrome due to an inborn error of cholesterol synthesis [32]. Although the SLOS phenotype includes cognitive deficits, unlike NPC1, these are not due to

neurodegeneration. Obtaining true pediatric CSF samples is precluded by ethical and statutory restrictions on research. Whereas we had no control over the collection and handling of the anonymized PLC samples, the SLOS samples were collected, handled, and processed identically to the NPC1 samples. Comparison of samples from the two comparison groups showed no significant difference with respect to total Tau levels ($p = 0.94$, Mann–Whitney-U test).

5.2 | Clinical Data and Biospecimen Collection

Clinical assessment included detailed history and physical exam. Clinical assessments also included formal swallowing evaluation and neurocognitive testing. Age of neurological onset was determined from clinical histories. Both the 17-domain and 5-domain NPC Neurological Severity Scores were determined based on clinical assessment as previously described [18, 19]. ASIS was determined by dividing the 17-domain NPC NSS by corresponding age in years [21]. NPC1 and non-NPC1 CSF samples were collected by lumbar puncture, aliquoted, frozen, and stored at −80°C. Due to limitations on obtaining true control pediatric samples, we used several types of CSF as comparison samples. These included anonymized pediatric laboratory controls (PLC) that were residual CSF samples collected for a clinically indicated procedure. Since we had no control over the collection and processing of these samples, we also used CSF obtained from individuals with SLOS (NCT00001721) collected and processed similarly to those obtained from individuals with NPC. We did not observe any significant differences between the PLC and SLOS comparison samples for total Tau ($p = 0.96$, Mann–Whitney-U test). Plasma samples were collected at times of visit, aliquoted, and stored at −80°C. Plasma from 21 NPC1 individuals (16.9 ± 13.5 years) and 25 control individuals (9.3 ± 3.2 years) were assayed for pTau(217) as described below.

5.3 | Total Tau and Phospho-Tau Assays

A Neuro3-plex assay (Simoa^R) was used to measure CSF levels of total Tau on a Quanterix SR-X Biomarker Detection System (Quanterix, Billerica, MA, USA). CSF samples were assayed in duplicate after dilution with sample diluent and assayed per the manufacturer's protocol. Both low-and high-range control samples were included on all 10 plates. Weighted averages of the inter-assay coefficients of variation (CV%) were 8.0% for the low-range control and 12.8% for the high-range control over the 10 plates. CSF pTau(181) was measured using a Simoa^R pTau-181 AdvantageV2 kit on the SR-X system. Plasma pTau(217) was measured using the Simoa^R ALZpath p-Tau 217 Advantage PLUS Reagent kit on a Quanterix HD-X Biomarker Detection System.

5.4 | Statistical Analysis

When applicable, descriptive statistics included frequencies and percentages for categorical variables, mean ± standard deviation, median and interquartile range, and range for numerical variables. Two sample Mann–Whitney U tests were performed to compare the different NPC1 cohorts and the non-NPC1 comparison group, and a Kruskal–Wallis test was used to compare age cohorts (GraphPad Prism).

For comparison of baseline values with ANO, ASIS, 17-domain NPC NSS, and 5-domain NPC NSS, Spearman correlation coefficients with 95% confidence intervals and p-values were calculated. For longitudinal analysis, a linear mixed effects model was used to determine the longitudinal effect of six fixed effect covariates (miglustat therapy (yes vs. no), intrathecal VTS270/adrabetadex therapy (yes vs. no), sex (male vs. female), baseline age (years), time elapsed since the baseline visit (years) and age of neurological onset (years)) on $\log_{10}$ transformed analyte levels. Exponentiated estimated coefficients and 95% confidence intervals quantify the effects of the covariates on the outcomes.

Adjusted p-values were obtained using a false discovery rate (FDR) procedure for multiplicity correction for both the baseline Spearman correlations and the linear mixed effects model analysis [33].

Author Contributions

N.X.C.: experimental design; data collection; data analysis; supervision; manuscript editing. **R.Z.:** data analysis, manuscript writing. **N.M.F.:** experimental design; data collection; supervision; manuscript editing. **J.I.:** data analysis. **D.M.A.:** data collection, supervision, manuscript editing. **R.A.L.:** data collection. **C.J.P.:** data collection. **H.O.M.:** data collection. **O.K.A.:** data collection. **K.P.R.:** data collection. **A.D.D.:** experimental design; manuscript editing. **E.B.-K.:** experimental design; funding; manuscript editing. **S.M.C.:** experimental design; funding; manuscript editing. **F.L.:** data analysis; supervision; funding; manuscript editing. **F.D.P.:** experimental design; data analysis; supervision; funding; manuscript editing.

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Ethics Statement

Clinical assessments and biomaterial reported in this paper were obtained in protocols initially approved by the NICHD IRB with ongoing approval by the NIH Clinical Center IRB (FWA00005897). Collection of samples provided by RUMC was approved by the RUMC IRB. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Consent

Written informed consent was obtained from participants or guardians.

Conflicts of Interest

Niamh X. Cawley, Ruyu Zhou, Nicole M. Farhat, James Iben, Derek M. Alexander, Rachel A. Luke, Cameron J. Padilla, Hibaaq O. Mohamed, Orsolya K. Albert, Kendall P. Robbins, Samar Rahhal, An Dang Do, Fang Liu, and Stephanie M. Cologna declare that they have no conflicts of interest. Elizabeth Berry-Kravis has received funding from Acadia, Alcobra, AMO, Asuragen, Avexis, Biogen, BioMarin, Cydan, Engrail,

Erydel, Fulcrum, GeneTx, GW, Healx, Ionis, Jaguar, Kisbee, Lumos, Marinus, Mazhi, Moment Biosciences, Neuren, Neurogene, Neurotrope, Novartis, Orphazyme/Kempharm/Zevra, Ovid, PTC Therapeutics, Retrophin, Roche, Seaside Therapeutics, Taysha, Tetra, Ultragenyx, Yamo, Zynerba, and Vtesse/Sucampo/Mallinckrodt Pharmaceuticals to consult on trial design or run clinical or lab validation trials in genetic neurodevelopmental or neurodegenerative disorders, all of which is directed to RUMC in support of rare disease programs; Dr. Berry-Kravis receives no personal funds, and RUMC has no relevant financial interest in any of the commercial entities listed. Forbes D. Porter has received research support via a Cooperative Research and Development Agreement between Vtesse/Sucampo/Mallinckrodt/Mandos Health and NICHD, NIH, to support the development of 2-HPβCD for the treatment of NPC.

Data Availability Statement

Underlying data are either included in this published article or available to IRB-approved researchers upon request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.