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Neurofilament Light and Its Association With CNS Involvement in Patients With Classic Infantile Pompe Disease

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Abstract

Background and objectives: Enzyme replacement therapy (ERT) has substantially improved the outcome of classic infantile Pompe disease, an inheritable muscle disease previously fatal at infancy. However, under treatment, patients develop white matter abnormalities (WMA) and neurocognitive problems. Therefore, upcoming therapies also target the brain. Currently biomarkers reflecting CNS involvement are lacking. We aimed to study the association of Neurofilament Light and CNS involvement.

Methods: To investigate the potential of NfL, we analysed serum samples of patients with classic infantile Pompe disease who were treated with ERT. The samples were collected at ages of <1, 5 and 10 years, as well as around MRI scans. We compared the outcomes to levels in age and sex matched peers. Control samples were originally collected as part of routine blood work in children that underwent small surgeries and stored in the biobank of the Erasmus MC/ Sophia Children's Hospital.

Results: We analysed 74 serum samples of 17 patients collected at ages ranging from 22 days to 21.2 years (1-8 samples per patients) and compared these to outcomes of 71 matched peers.

In the first year of age NfL levels in patients and controls were similar (10.3 vs. 11.0 pg/ml), but mixed linear model analysis showed a yearly increase of NfL of 6.0% in patients, compared to a decrease of 8.8% in controls ($p < 0.001$). Higher NfL was associated with lower IQ scores ($p = 0.009$) and lower processing speed scores ($p = 0.001$).

Discussion: We found significant differences in NfL levels between patients and controls, and a good association between NfL and cognition. NfL deserves further exploration as a biomarker for CNS involvement in patients with classic infantile Pompe disease.

Introduction

Pompe disease, also known as Glycogen Storage Disease type 2 (GSDII) is a metabolic myopathy caused by the lysosomal deficiency of acid alpha-glucosidase (GAA). This deficiency leads to the accumulation of glycogen most prominently in muscle cells,[1,2] but to a lesser extent also in glial cells, astrocytes, and neurons in the central nervous system.[3,4]

Patients with the classic infantile phenotype of Pompe disease have no residual enzyme activity and consequently, manifest the most severe phenotype of the disease. Untreated, these patients die before the age of one year due to cardiorespiratory failure.[1,5,6] The approval of enzyme replacement therapy (ERT) in 2006 has significantly improved the survival of these patients and enabled them to achieve formerly unmet motor milestones such as the ability to stand and walk.[7-9] Globally, the first treated patients with classic infantile disease are now in their twenties.[10,11]

Neurocognitive development of ERT-treated patients is normal during the first years of life. However, in childhood and adolescence, neuroimaging unveils slowly progressive white matter abnormalities (WMA), while cognitive tests show a decrease of processing speed and in a subset of patients a more generalized cognitive decline .[10,12-14] These symptoms are thought to be the result of the inability of ERT to cross the blood-brain barrier and subsequent accumulation of glycogen. This is also illustrated by the fact that autopsy studies in ERT-treated patients have shown glycogen accumulation in the brain.[15] New treatments for classic infantile Pompe disease should therefore include the brain as an additional target.

To appreciate the effect of innovative therapies in upcoming phase 1-2 studies, it is important to fully understand the variability of CNS involvement in patients with classic infantile Pompe disease. Up till now the extent and rate of CNS involvement has been reported in a limited number of small cohorts of classic infantile patients and ranges from normal and stable to extensive and fast progressive[10,12-14] . The methods currently used to monitor central nervous system (CNS) involvement have disadvantages. MRI scans are expensive and burdensome as these often require anaesthesia. The applied neuropsychological tests vary and are advised not to be performed more than once every one to two years due to a potential learning effect. In addition, both methods do not show evident deviations until later in life later in life[10,12]. Although, other authors describe a correlation between CK, urinary tetra-

saccharides and motor response to ERT [16], there are no early markers yet that reflect CNS damage. Body fluid biomarkers could be a cheap and easy to obtain alternative.

One such potential biomarker is Neurofilament Light (NfL). Neurofilament is a main protein of the axonal cytoskeleton which consists of different subunits, of which NfL is the most abundant. NfL can be measured in cerebral spinal fluid (CSF), but can also be reliably measured in blood nowadays, through Single Molecule Array (Simoa). Levels of NfL in serum, which is more readily available than CSF, are highly correlated with levels in CSF.[17,18] Increased levels of NfL are associated with neuroaxonal and astroglial damage and correlate with disease activity in many paediatric neurological diseases, including Spinal Muscular Atrophy (SMA), Ceroid Lipofuscinosis type 2 (CLN2), Metachromatic Leukodystrophy (MLD), and X-linked Adrenoleukodystrophy (X-ALD).[18-23] Unfortunately reference values of NfL in both serum and CSF for the paediatric populations are lacking.

We hypothesize that NfL is increased in patients with classic infantile Pompe disease and may be correlated to clinical outcomes. In this study, we aimed to determine the potential of NfL as a biomarker for CNS involvement in patients with classic infantile Pompe disease, by comparing NfL levels in patients to age and sex matched controls, and by investigating the correlation of NfL levels with WMA on MRI and cognitive outcomes.

Materials and Methods

Patients With Classic Infantile Pompe Disease

All patients with classic infantile Pompe disease in the Netherlands are enrolled in a prospective standardized follow-up study. From this cohort, we included all patients who had an MRI brain scan between 1999 and September 19, 2020 (database lock). Classic infantile

Pompe disease was defined as symptom onset before the age of 6 months, presence of a hypertrophic cardiomyopathy, profound enzyme deficiency (<1% of normal values in fibroblasts or below reference values in leukocytes) and two severe pathogenic variants in the GAA gene (www.pompevariantdatabase.nl). [24, 25]

Patients were treated with alglucosidase alfa, at doses ranging from 20 mg/kg/2 weeks to 40 mg/kg/week [8].

As per protocol blood samples were taken twice to four times a year, cognitive evaluations were done once every 1-2 years, and MRI scans were performed at regular intervals every 1-3 years after 2016 and before 2016 occasionally. We analysed NfL levels in blood at set time points (at <1 year of age, if available before start of ERT and otherwise as close to start as possible , as well as at age 5 and 10 years) and around the time an MRI scan was available. We allowed for a window of one year difference between the time points at which the different measurements (Nfl, cognitive tests and MRI scans) were taken.

Control Samples

Serum samples from age and sex matched controls were obtained from the biobank of the Sophia Children's Hospital. These were taken as part of routine blood work in children that underwent small surgeries.

NfL Analysis Using Simoa Technique

Blood samples were stored in serum tubes at -80 degrees Celsius at the Erasmus Medical Centre's Biobank until use. 250 µL serum was analysed in the Neurochemistry Lab of the department of Clinical Chemistry at the Amsterdam University Medical Centres (AUMC),

using the Simoa technique (Simoa HD-1) and the NF-light Kit according to the instructions. [26] Before measurement, samples were centrifuged for 10 minutes at room temperature at 1800g. Measurements were performed by blinded, certified technicians, in singlicate. Values are expressed in picogram per millilitre (pg/ml).

MRI Scans

We made MRI scans of the brain using a 1.5T or 3T system (EchoSpeed; GE Healthcare, Milwaukee, WI, USA), and a dedicated 8-channel head coil. MRIs were conducted according to a standardized protocol consisting of T1-weighted, T2-weighted, and fluid-attenuated inversion recovery images.[10] MRIs were graded using the method described by Ebbink et al. (2018),[10] recognizing four stages of involvement. Stage 0: no abnormalities. Stage 1: periventricular white matter (PVWM) involvement around the centrum semiovale. Stage 2: additional abnormalities in the subcortical white matter, internal capsule, external capsule and corpus callosum. Stage 3: extension to the u-fibres, basal ganglia, corticospinal tract, and/or infra-tentorial white matter. All MRIs were blinded, and independently graded by a paediatric neuro-radiologist (MD), a paediatric neurologist (JH), and a PhD student (JD). Discrepancies were solved by a consensus meeting.

Cognitive Tests

For patients aged ≤ 6 years we used the Griffiths Mental Developmental Scales until the end of 2017 [27] and the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III-NL) after 2017.[28]

For children aged 6 years to 16 years and 11 months, we used the Wechsler Intelligence Scales for Children, third or fifth edition ((WISC-III-NL or WISC-V-NL) [29,30] and for patients older than 16 years and 11 months the Wechsler Adult Intelligence Score IV-NL (WAIS-IV-NL) was used.[31] All neuropsychological tests measured either full scale intelligence quotients (IQ) or developmental quotients (DQ), while processing speed (PS) could be tested with the WPPSI, WISC and WAIS. The scores of all tests were compared to the normative data of the Dutch population. Mean score for the tests is 100, with a standard deviation (SD) of 15 points. A score above 85 is indicative of a normal development, a score between 84 and 70 of mild developmental delay, and a score below 70 of intellectual disability.

Statistical Analyses

Statistical analyses were performed using R, version 4.1.3 (2022-03-10, "One Push-Up")
To account for the correlations between repeated measurements in individual patients, we used linear mixed-models. Interaction terms between models were tested using the likelihood-ratio test. Models' assumptions were checked using residual plots. To correct for skewness of data, NfL levels were log-transformed. We used a mixed effects continuation ratio model for the ordinal data of the MRI brain scans.
The "nlme" and "GLMM adaptive" packages were used for the linear mixed-effects and continuation ratio models, the "lmertTest" package was used for testing of interaction terms. Descriptive statistics, including median and range, were used to summarize demographic and clinical data. A p-value of <0.05 was assumed to be significant.

Standard Protocol Approvals, Registrations, and Patient Consents

Study protocols of studies in patients with Pompe disease and controls were approved by the Erasmus MC Medical Ethical Review Committee. Written informed consent was obtained from the patients as well as of controls and/ or their parents.

Data Availability

Unpublished anonymized data within this article are available on reasonable request from a qualified investigator.

Results

General Characteristics

Seventeen patients with classic infantile Pompe disease were included (Table 1). Four (24%) patients were Cross-reactive immunological material (CRIM) negative, twelve (71%) were CRIM positive and one patients' CRIM status was unknown. Median age at diagnosis was 2.3 months (range: 3 days - 5.7 months).

All patient were treated with recombinant human GAA (rhGAA). Their median age at start of ERT was 2.6 months (range: 4 days – 5.8 months). Median age at last NfL sample was 9.1 years (3.6 – 21.2 years), 4.8 years (range: 3.6 – 7.7) for CRIM negative patients and 9.9 years (range: 5.4 - 21.2) for CRIM positive patients. Additional clinical data can be found in Table 1.

NfL Levels in Patients and Controls

NfL blood levels were determined as close as possible to start of ERT (age <1) and at 5, and 10 years of age as well as at the time of MRI scans (Table 1). Five patients had baseline blood samples taken prior to initiation of ERT (age range: 0.2 – 0.4 years), ten had baseline samples taken thereafter (age range: 0.1 – 0.9 years), while two patients had no baseline sample available before the age of 1 year. Median NfL was 12.4 pg/ml (range 6.7 – 19.0) for ERT-naïve patients and 9.4 pgram/ml (5.5 – 21.5) in patients with a baseline sample after the start of ERT.

A linear mixed effects model analysis including all samples (*n=74*) of the 17 patients (age range: 22 days - 21.2 years, 1 - 8 samples per patient) and samples of 71 unaffected age and sex matched controls (age range: 1.8 months – 16.1 years) showed that NfL levels in control patients decreased with 8.8% yearly, *[95% CI 6.4, 11.2]* while these increased in Pompe patients with 6.0% yearly, *[95% CI 3.4, 8.0]*. The difference between these two groups was significant ($p < 0.001$).

In our population, CRIM negative patients (open triangles) had 45%, ***[****95 CI 8.4, 67.2]*, lower NfL levels compared with CRIM positive patients (Figure 1; closed circles). This difference was maintained after correction for age ($p = 0.028$).

Figure 1 shows all individual NfL samples and the locally estimated scatterplot smoothing (LOESS) regression curve as well as the 95%-confidence interval (shadows). The Figure shows that there is inter-individual and intra-individual variation.

Selection of three times points (age < 1 year, 5 and 10 years) showed the following. At first NfL assessment (age <1 year) the median NfL value in patients was 10.3 (range: 5.4 – 21.5,

n=15) pg/ml and 11.0 (6.7 – 49.4, *n=15*) pg/ml in controls; at age 5 years the median was 7.7 (4.5 – 21.3, *n=16*) pg/ml in patients and 6.4 (4.1 – 14.4, *n=16*) pg/ml in controls and at 10 years the median NfL value was 18.6 (5.2 - 41.1, *n=8*) and 9.8 (8.9 - 10.7, *n=8*) pg/ml in controls.

Association Between NfL and Brain Involvement on MRI

Forty-three MRI scans of 16 patients (age range: 2.7 - 21.2 years, 1 - 6 MRIs per patient) could be paired with 43 NfL blood samples that were taken around the same time (Figure 2). One patient with one MRI scan was excluded due to the unavailability of 3D sequences. One MRI scan was normal (stage 0; age 7.6 years). Six MRI scans were graded as stage 1 (median age: 5.4 years (2.7 – 7.5 years)). Nineteen scans were graded as stage 2 (median age: 9.1 years (4.6 – 12.9 years)), and 17 MRI scans were graded as stage 3 (median age: 13.2 years (4.4 – 21.2 years)).

The NfL level of the patient with a normal MRI was 4.4 pg/ml. The median NfL of patients with stage 1 was 9.3 pg/ml (range: 6.1 – 97.4), 16.6 pg/ml (range: 5.3 – 37.6) of those with stage 2, and 21.3 pg/ml (range: 6.7 - 47.8) of patients with stage 3. Due to the instability of the mixed-effects continuation ratio model, the differences between the different groups could not be tested statistically.

Association Between NfL and Cognitive Outcomes

We compared NfL levels and cognitive scores (Figure 3). Forty-seven serum samples of 16 patients could be paired to 47 total IQ/DQ scores (age range: 0.16 – 21.2 years, 1-5 tests per patient), and 25 samples to 25 results of PS scTabores (age range: 5.3 – 21.2 years, 1-4 tests

per patient). A doubling of NfL corresponded to a decrease of 7.7 IQ points, *[95% CI -13.2, -2.1, p = 0.009),* and a decrease of 13.9 PS score points, *[95% CI -20.8, -7.0, p = 0.001).*

Discussion

We found that NfL levels increased in patients with classic infantile Pompe disease from age 0 to 21.2 years, whereas these decreased over time in healthy, age and sex matched controls at group level. In the first year of life, NfL levels were similar in both groups. After the age of 5 years, Nfl levels of controls and patients deviated, NfL levels increased 6.0% per year in patients with Pompe disease, while they decreased by 8.8% per year in controls. With increasing NFL levels a significant decrease of IQ and PS scores was observed. Although there seemed to be a trend, no statistical relationship could be measured between NfL levels and MRI brain scores.

Cross-reactive immunological material (CRIM) negative patients, who produce no native GAA protein are thought to have a poorer prognosis than CRIM positive patients, who produce some inactive GAA protein. In our study, CRIM negative patients did not have higher NfL levels than CRIM positive patients, even after correction for age. As the number of CRIM negative patients (*n=4*) in our study was low and their age young, this finding should be interpreted with caution.

Increased levels of NfL are predominantly found in diseases with neuroaxonal or astroglial injury. Therefore we hypothesize that the gradual increase of NfL in patients with classic infantile Pompe disease is the biochemical result of progressive glycogen accumulation and/ or damage to the CNS, leading to leakage of NfL into CSF and serum.[12] The elevation of NfL reflects the fact that ERT cannot pass the blood brain barrier and thus cannot reach glial cells, astrocytes, and neurons, where glycogen deposition has been found in autopy studies.[3,4]

Innovative therapies could provide a solution for the still unmet medical needs in classic infantile Pompe disease. Hematopoietic stem cell (HSPC)-mediated lentiviral gene therapy (LVGT) resulted in normalization of glycogen levels and neuro-inflammation in the brain of Pompe knock-out mice[32,33]. In other LSDs such as MPS I, II and CLN2 alternative approaches are effective such as intra-cerebral-ventricular ERT or IgG-ERT fusion proteins, where the IgG domain is a receptor-specific monoclonal antibody (MAb) that targets an endogenous BBB receptor transporter.[34,35] . The findings from our study indicate that Nfl might be suitable as a biomarker both for follow-up and evaluation of emerging therapies that include the brain as a target.

NfL Course in Individual Patients

While Nfl levels increased with age in our cohort of patients with classic infantile patients on group level, we also observed inter-individual and intra-individual NfL variations, for which no clear explanation could be found. Similar unexplained intra-individual variations are also reported in other diseases such as CLN2 and MLD. More data, preferably in larger cohorts, are needed to provide context on the variability observed.

Nfl levels are also elevated in case of a peripheral neuropathy. A recent study in patient with classic infantile Pompe disease who learned to walk reported distal muscle weakness[36]. The pathophysiological process underlying the distal muscle weakness is not yet elucidated. This might be myopathic, neuropathic or a combination of the both. [37,38] NfL increases in this cohort therefore might potentially partly be explained by peripheral neuropathy.

Comparison to Other Lysosomal Storage Disorders

The highest NfL level measured in our cohort of patients with classic infantile Pompe disease was 97.4 pg/ml. This was measured in a patient aged 4.1 years. In the oldest patient, aged 21.2 years, NFL level was measured at29.2 pg/ml. . These NfL levels were both considerably lower than those found in the more rapidly progressive MLD and CLN2.[18,20] In these diseases, NfL levels were already high (measured around 100 - 300 pg/ml) around the time of diagnosis. Median age at diagnosis was 11.1 years in the study of MLD patients and 4.3 years in the study on CLN2 patients. In the study of MLD, NfL decreased in both treated and untreated patients. This was explained by the rapid progression of CNS damage and a concomitant rapid reduction of healthy brain tissue as a source of NfL release into serum. The decrease in treated and untreated patients with MLD complicates the interpretation of the effect of therapy using NfL as a biomarker.[20] Our data show that the CNS progression in Pompe disease is different. Since the CNS involvement in Pompe disease is much slower, and NfL increased in our study over time from childhood through young adulthood in the absence of CNS-targeting therapy, we conclude that NfL holds promise as a biomarker to evaluate the effects of innovative future treatments targeting the brain.

Nfl in Healthy Age and Sex Matched Controls

In our study the Nfl levels in controls were relatively high during the first year and showed a decline thereafter during childhood and adolescence. It has been shown by others that during late adulthood Nfl starts to increase again. The decline of Nfl during childhood with increasing age was also observed by others and has been hypothesized to be related to factors such as ongoing myelination during the first years of life. [20,39]

Strength and imitations

The strength of our study is the longitudinal follow-up combining NfL levels with cognitive tests and neuroimaging as well as the inclusion of a paediatric reference cohort.

A limitation is the small size of our cohort, due to the rarity of Pompe disease. Studies in larger groups of patients could provide additional insight in the role of factors such as CRIM status and enable statistical confirmation of the trends we observed between increased NfL levels and MRI brain scan scores. Additional these could provide further insight in the levels of NfL in patients with classic infantile Pompe disease in the first year of life, clarify at what age between the 5 and 10 years Nfl levels in patients deviate significantly from normal and elucidate the extent and cause of inter- and intra-individual variations.

Conclusion

In conclusion, we found a significant difference between NfL levels of ERT treated patients with classic infantile Pompe disease and controls at group level during follow-up from infancy to young adulthood. In addition, we found that NfL levels were increased in patients with lower IQ and processing speed scores. This study indicates that NfL is a promising biomarker for CNS involvement in classic infantile Pompe disease and deserves additional research as a biomarker to monitor the effect of emerging treatments targeting the brain.

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WNL-2023-000312_etab1 ---http://links.lww.com/WNL/C909

References

1. van der Ploeg AT, Reuser AJ. Pompe's disease. *Lancet*. Oct 11 2008;372(9646):1342-53.
2. Reuser AJ, Hirschhorn R, Kroos MA. *Pompe disease: Glycogen storage disease type II, Acid alpha-glucosidase (acid maltase) deficiency*. McGraw Hill: The Online Metabolic & Molecular Bases of Inherited Disease; 2018.
3. Clement DH, Godman GC. Glycogen disease resembling mongolism, cretinism, and amytonia congenita; case report and review of literature. *J Pediatr*. Jan 1950;36(1):11-30, illust.
4. Gambetti P, DiMauro S, Baker L. Nervous system in Pompe's disease. Ultrastructure and biochemistry. *J Neuropathol Exp Neurol*. Jul 1971;30(3):412-30.
5. Kishnani PS, Hwu WL, Mandel H, et al. A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease. *J Pediatr*. May 2006;148(5):671-676.
6. van den Hout HM, Hop W, van Diggelen OP, et al. The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature. *Pediatrics*. Aug 2003;112(2):332-40.
7. Ditters IAM, Huidekoper HH, Kruijshaar ME, et al. Effect of alglucosidase alfa dosage on survival and walking ability in patients with classic infantile Pompe disease: a multicentre observational cohort study from the European Pompe Consortium. *Lancet Child Adolesc Health*. Jan 2022;6(1):28-37.
8. Poelman E, van den Dorpel JJA, Hoogeveen-Westerveld M, et al. Effects of higher and more frequent dosing of alglucosidase alfa and immunomodulation on long-term clinical outcome of classic infantile Pompe patients. *J Inherit Metab Dis*. Nov 2020;43(6):1243-1253.
9. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology*. Jan 9 2007;68(2):99-109.
10. Ebbink BJ, Poelman E, Aarsen FK, et al. Classic infantile Pompe patients approaching adulthood: a cohort study on consequences for the brain. *Dev Med Child Neurol*. Jun 2018;60(6):579-586.
11. Van den Hout H, Reuser AJ, Vulto AG, Loonen MC, Cromme-Dijkhuis A, Van der Ploeg AT. Recombinant human alpha-glucosidase from rabbit milk in Pompe patients. *Lancet*. Jul 29 2000;356(9227):397-8.
12. Korlimarla A, Lim JA, Kishnani PS, Sun B. An emerging phenotype of central nervous system involvement in Pompe disease: from bench to bedside and beyond. *Ann Transl Med*. Jul 2019;7(13):289.
13. Paoletti M, Pichiecchio A, Colafati GS, et al. Multicentric Retrospective Evaluation of Five Classic Infantile Pompe Disease Subjects Under Enzyme Replacement Therapy With Early Infratentorial Involvement. *Front Neurol*. 2020;11:569153.
14. Spiridigliozzi GA, Keeling LA, Stefanescu M, Li C, Austin S, Kishnani PS. Cognitive and academic outcomes in long-term survivors of infantile-onset Pompe disease: A longitudinal follow-up. *Mol Genet Metab*. Jun 2017;121(2):127-137.
15. Pena LD, Proia AD, Kishnani PS. Postmortem Findings and Clinical Correlates in Individuals with Infantile-Onset Pompe Disease. *JIMD Rep*. 2015;23:45-54.
16. Chien YH, Tsai WH, Chang CL, et al. Earlier and higher dosing of alglucosidase alfa improve outcomes in patients with infantile-onset Pompe disease: Evidence from real-world experiences. *Mol Genet Metab Rep*. Jun 2020;23:100591.
17. Bridel C, van Wieringen WN, Zetterberg H, et al. Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. *JAMA Neurol*. Sep 1 2019;76(9):1035-1048.
18. Ru Y, Corado C, Soon RK, Jr., et al. Neurofilament light is a treatment-responsive biomarker in CLN2 disease. *Ann Clin Transl Neurol*. Dec 2019;6(12):2437-2447.

19. van Ballegoij WJC, van de Stadt SIW, Huffnagel IC, et al. Plasma NfL and GFAP as biomarkers of spinal cord degeneration in adrenoleukodystrophy. *Ann Clin Transl Neurol*. Nov 2020;7(11):2127-2136.
20. Beerepoot S, Heijst H, Roos B, et al. Neurofilament light chain and glial fibrillary acidic protein levels in metachromatic leukodystrophy. *Brain*. Mar 29 2022;145(1):105-118.
21. Bhalla A, Ravi R, Fang M, et al. Characterization of Fluid Biomarkers Reveals Lysosome Dysfunction and Neurodegeneration in Neuronopathic MPS II Patients. *Int J Mol Sci*. Jul 22 2020;21(15)
22. Weinhofer I, Rommer P, Zierfuss B, et al. Neurofilament light chain as a potential biomarker for monitoring neurodegeneration in X-linked adrenoleukodystrophy. *Nat Commun*. Mar 22 2021;12(1):1816.
23. Nitz E, Smitka M, Schallner J, et al. Serum neurofilament light chain in pediatric spinal muscular atrophy patients and healthy children. *Ann Clin Transl Neurol*. Oct 2021;8(10):2013-2024.
24. Viamonte MA, Filipp SL, Zaidi Z, Gurka MJ, Byrne BJ, Kang PB. Phenotypic implications of pathogenic variant types in Pompe disease. *J Hum Genet*. Nov 2021;66(11):1089-1099.
25. Niño MY, Wijgerde M, de Faria DOS, et al. Enzymatic diagnosis of Pompe disease: lessons from 28 years of experience. *Eur J Hum Genet*. Mar 2021;29(3):434-446.
26. Neurofilament Light. Quanterix. Accessed 06-oct-2022, 2022. https://www.quanterix.com/simoa-assay-kits/nf-light/
27. Griffiths R. *The abilities of young children: A comprehensive system of mental measurement for the first eight years of life*. Association for Research in Infant and Child Development; 1984.
28. Wechsler D, Hendriksen J, Hurks P. *WPPSI-III-NL. Nederlandstalige bewerking. Afname en Scoringshandleiding.* Amsterdam: Pearson Assessment and Information B.V.; 2009.
29. Kort W, Schittekatte M, Dekker PH. *WISC-III NL Wechsler Intelligence Scale for Children. Derde Editie NL. Handleiding en Verantwoording*. Amsterdam: Harcourt Test Publishers/Nederlands Instituut voor Psychologen; 2005.
30. Wechsler D. *Wechsler Intelligence Scale for Children, vijfde editie; Nederlandstalige bewerking*. Amsterdam: Pearson Benelux B.V.; 2018.
31. Wechsler D. *Wechsler Adult Intelligence Scale, vierde editie; Nederlandstalige bewerking.* Amsterdam: Pearson Benelux B.V.; 2012.
32. Liang Q, Catalano F, Vlaar EC, et al. IGF2-tagging of GAA promotes full correction of murine Pompe disease at a clinically relevant dosage of lentiviral gene therapy. *Mol Ther Methods Clin Dev*. Dec 8 2022;27:109-130.
33. Liang Q, Stok M, van Helsdingen Y, et al. Lentiviral Stem Cell Gene Therapy for Pompe Disease. *J Neuromuscul Dis*. 2015;2(s1):S64.
34. Giugliani R, Giugliani L, de Oliveira Poswar F, et al. Neurocognitive and somatic stabilization in pediatric patients with severe Mucopolysaccharidosis Type I after 52 weeks of intravenous brain-penetrating insulin receptor antibody-iduronidase fusion protein (valanafusp alpha): an open label phase 1-2 trial. *Orphanet J Rare Dis*. Jul 5 2018;13(1):110.
35. Okuyama T, Eto Y, Sakai N, et al. A Phase 2/3 Trial of Pabinafusp Alfa, IDS Fused with Anti-Human Transferrin Receptor Antibody, Targeting Neurodegeneration in MPS-II. *Mol Ther*. Feb 3 2021;29(2):671-679.
36. van den Dorpel JJA, Poelman E, Harlaar L, et al. Distal muscle weakness is a common and early feature in long-term enzyme-treated classic infantile Pompe patients. *Orphanet J Rare Dis*. Sep 14 2020;15(1):247.
37. Tsai LK, Hwu WL, Lee NC, Huang PH, Chien YH. Clinical features of Pompe disease with motor neuronopathy. *Neuromuscul Disord*. Nov 2019;29(11):903-906.
38. Schänzer A, Görlach J, Claudi K, Hahn A. Severe distal muscle involvement and mild sensory neuropathy in a boy with infantile onset Pompe disease treated with enzyme replacement therapy for 6 years. *Neuromuscul Disord*. Jun 2019;29(6):477-482.

39. Bishop DL, Misgeld T, Walsh MK, Gan WB, Lichtman JW. Axon branch removal at developing synapses by axosome shedding. *Neuron*. Nov 18 2004;44(4):651-61.

Table 1 Characteristics of the classic infantile Pompe patients

No. of patients (%female)	17 (47%)
CRIM status; positive/negative/unknown	12/4/1
	Median (range)
Age at diagnosis	2.3 months (3 days - 5.7 months)
Age at start ERT	2.6 months (4 days - 5.8 months)
Age at sample <1 year	4.6 months (22 days - 11.1 months)[1]
Age at last NfL sample	9.1 years (3.6 - 21.2 years)
No. of blood samples used for NfL measurement per patient	4 (1 - 8)
No. of cognitive tests per patient	2 (0 - 6)
Age at first IQ/DQ measurement	7.2 months (1.9 months - 10.6 years)[2]
Age at last IQ/DQ measurement	8.0 years (5.8 months - 21.2 years)[3]
IQ/DQ at first NfL assessment	89 (64 - 113)[3]
IQ/DQ at last NfL assessment	66 (45 - 102)[3]
Age at first PS measurement	6.8 years (5.3 - 10.6 years)[3]
Age at last PS measurement	8.5 years (5.3 - 21.2 years)[3]
PS at first cognitive assessment	89 (55 - 111)[3]
PS at last cognitive assessment	70 (45 - 111)[3]
Age at first MRI measurement	6.3 years (2.7 - 13.7 years)[4]
Age at last MRI measurement	8.7 years (4.6 - 21.2 years)[4]
No. of patients that died (at age)	3 (4.4; 14.5 and 15.5 years)

Figure legends

Table 1 Characteristics of the classic infantile Pompe patient study population

Neurofilament light = NfL; full scale intelligence quotient (IQ); developmental quotient (DQ); processing speed (PS)

Outcomes are presented as medians with ranges between brackets. [1] Sample not available for two patients. [2] No cognitive test data available for 1 patient. [3] Processing speed (PS) scores not available for 5 patients. [4] MRI brain scan data not available for 1 patient.

Figure 1 NfL levels in classic infantile Pompe patients and controls

Neurofilament Light (NfL) levels in classic infantile Pompe patients (red) and controls (blue), visualized with locally estimated scatterplot smoothing (LOESS) curves and the 95%-confidence intervals (shadows). The y-axis represents NfL in pg/ml on a logarithmic scale, and the x-axis represents the age in years. Controls are represented by open circles, cross-reactive immunological material (CRIM) negative Pompe patients by triangle symbols, CRIM positive Pompe patients by closed circles, and the patient whose CRIM status is unknown is signified by a square symbol.

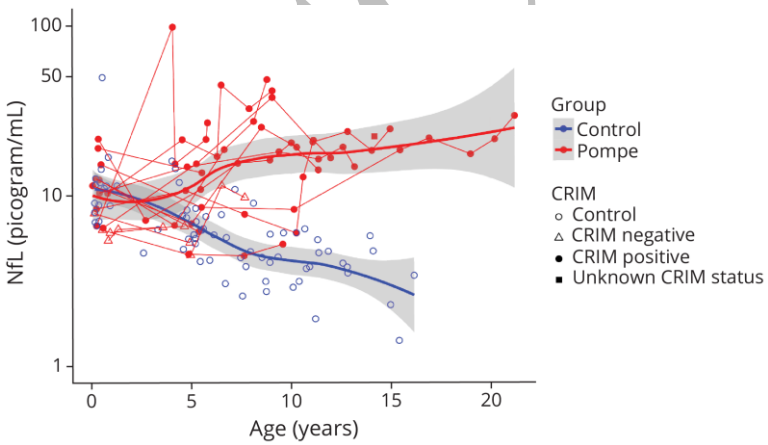


Figure 2 NfL levels expressed against MRI brain scores

Neurofilament Light (NfL) levels in classic infantile Pompe patients expressed against MRI brain scores. The y-axis represents NfL levels in pg/ml, and the x-axis the MRI brain score.

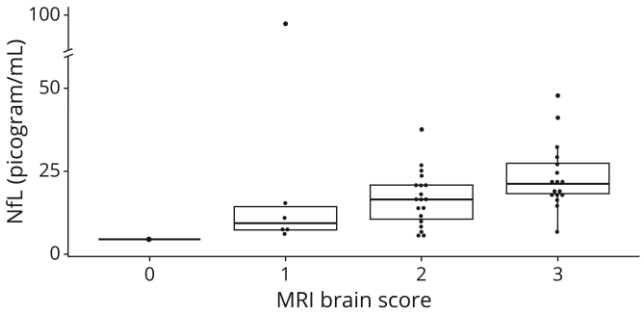
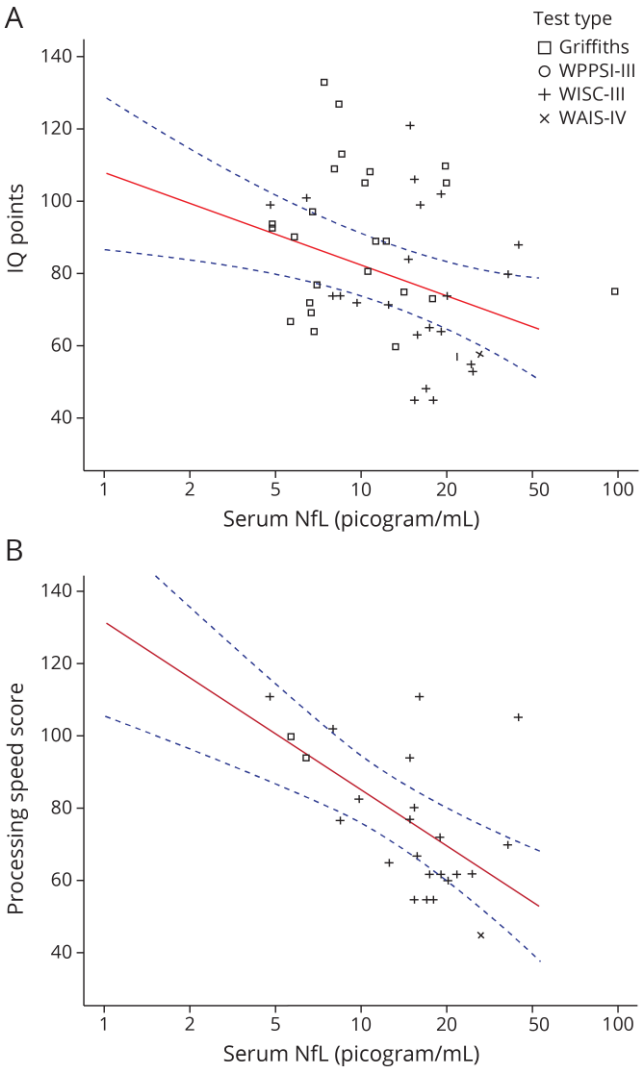


Figure 3 Association between repeated measures of (A) Total IQ, (B) Processing Speed (PS) scores expressed and Neurofilament Light (NfL) levels in blood of classic infantile Pompe patients.

The y-axis represents IQ/PS scores, and the x-axis represents NfL levels in pg/ml on a logarithmic scale. Circular symbols represent measurements of the Griffiths Mental Developmental scales, triangles represent Wechsler Adult Intelligence Scale IV (WAIS-IV), square symbols represent scores of the Wechsler Intelligence Scales for Children III (WISC-III), and plus-symbols represent measurements of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III-NL). The red continuous line represents the change in cognitive tests scores, with the blue dotted lines representing upper and lower limits..



Abbreviations:

CLN2 = ceroid lipofuscinosis type 2; CRIM = cross-reactive immunological material; DQ = developmental quotient; ERT = enzyme replacement therapy; GAA = acid alpha-glucosidase; GSDII = glycogen storage disease type II; IQ = full scale intelligence quotient; LSD = lysosomal storage disorder; MLD = metachromatic leukodystrophy; NfL = neurofilament light; PS = processing speed; PVWM = periventricular white matter; rhGAA = recombinant human acid alpha-glucosidase; SD = standard deviation; Simoa = single molecule array; SMA = spinal muscular atrophy; WAIS-IV = Wechsler adult intelligence score fourth edition; WISC-III/WISC-V = Wechsler intelligence scales for children third/fifth edition; WMA = white matter abnormalities; WPPSI = Wechsler preschool and primary scale of intelligence; X-ALD = X-linked adrenoleukodystrophy