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CNS, Lung, and Lymph Node involvement in Gaucher disease type 3 after 11 years of therapy: Clinical, Histopathologic, and Biochemical Findings

Thomas A Burrow^{#1,2}, Ying Sun^{#1,2}, Carlos E Prada^{1,4}, Laurie Bailey¹, Wujuan Zhang³, Amanda Brewer³, Steve W. Wu^{2,6}, Kenneth D R Setchell^{2,3}, David Witte^{2,3}, Mitchell B Cohen^{2,5}, and Gregory A Grabowski^{1,2}

¹Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati Ohio.

²Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati Ohio.

³Department of Pathology and Laboratory Medicine, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, Ohio.

⁴Centro de Medicina Genómica y Metabolismo, Fundación Cardiovascular de Colombia, Colombia.

⁵Division of Pediatric Gastroenterology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

⁶Division of Pediatric Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

These authors contributed equally to this work.

Abstract

A Caucasian male with Gaucher disease type 3, treated with continuous enzyme therapy (ET) for 11 years, experienced progressive mesenteric and retroperitoneal lymphadenopathy, lung disease, and neurological involvement leading to death at age 12.5 years. Autopsy showed significant pathology of the brain, lymph nodes, and lungs. Liver and spleen glucosylceramide (GluCer) and glucosylsphingosine (GluS) levels were nearly normal and storage cells were cleared. Clusters of macrophages and very elevated GluCer and GluS levels were in the lungs, and brain parenchymal and perivascular regions. Compared to normal brain GluCer (GC 18:0), GluCer species with long fatty acid acyl chains were increased in the patient's brain. This profile was similar to that in the patient's lungs, suggesting these lipids were present in brain perivascular macrophages. In the patient's brain, generalized astrogliosis, and enhanced LC3, Ubiquitin, and Tau signals were identified in the regions surrounding macrophage clusters, indicating proinflammation, altered

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Correspondence to: T. Andrew Burrow, M.D. Cincinnati Children's Hospital Medical Center Division of Human Genetics 3333 Burnet Avenue, MLC 4006 Cincinnati, OH 45229-3039 Phone: (513) 636-4760 Fax: (513) 636-7297 thomas.burrow@cchmc.org.

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autophagy, and neurodegeneration. These findings highlight the altered phenotypes resulting from increased longevity due to ET, as well as those in poorly accessible compartments of brain and lung, which manifested progressive disease involvement despite ET.

Keywords

Gaucher Disease; Lysosomal Storage Disease; Enzyme Replacement Therapy; lymphadenopathy; Pathology

1. Introduction

Gaucher disease is an autosomal recessive lysosomal storage disease caused by deleterious mutations in *GBA1* with resultant defective function of the expressed enzyme, acid β -glucosidase (glucocerebrosidase, GCase; EC 3.2.1.45). The consequent excess accumulation of glucosylceramide (GluCer) and glucosylsphingosine (GluS) in cells of monocyte/macrophage and other lineage cells leads to varying degrees of multi-organ involvement (1). Three phenotypes of Gaucher disease are classically described, based upon the presence or absence and severity of neurological involvement (1). Gaucher disease type 1, the non-neuronopathic variant, accounts for ~90% of cases of Gaucher disease in the Western world, with features usually limited to the visceral organs. Gaucher disease types 2 and 3 are neuronopathic variants, which are distinguished by the age of onset and rate of progression of neurological disease. In general, Gaucher disease type 2 (the acute neuronopathic variant) has early onset (i.e., birth to 6 months of life), severe, progressive neurological disease and visceral disease, followed by death within the first 1-2 years of life. Gaucher disease type 3 (the sub-acute neuronopathic variants) has later onset neurological and visceral disease of varying severity. Survival is often into the 3rd to 4th decades of life. The demarcation between the neuronopathic variants is imprecise, and it is more accurate to consider such phenotypes as a continuum of disease (1).

Intravenous (IV) infusion of recombinant GCase is a safe and effective treatment for the treatment of visceral manifestations of Gaucher disease. Because IV administered GCase does not cross the blood brain barrier in significant amounts, there is little if any, effect on CNS parenchymal involvement (2). Lung and lymph nodes also appear to have a diminished/absent response, indicating that certain organs are poorly accessible to IV enzyme therapy (ET) (2-4). The mechanistic basis for such poor access is not understood.

Here, the clinical course and post mortem histological and biochemical analyses are described 12.5-year-old male with Gaucher disease type 3, who had received 11 years of ET. Early findings in this patient have been reported. (3, 5, 6). The disease course was complicated by progressive massive mesenteric and retroperitoneal lymphadenopathy, infiltrative pulmonary disease, and neurological disease. These findings highlight the emerging phenotypic spectrum of patients treated with ET leading to the unexpected evolution of new manifestations of Gaucher disease, despite long-term ET.

2. METHODS

2.1 Consent

The parents provided informed consent for autopsy and histological and biochemical analyses of tissues as well as publication, in accordance with hospital and institutional review board policies.

2.2 Tissue collection and histological analyses

Tissues were harvested at autopsy, conducted approximately 8 hours after death. For lipid analyses, tissues were frozen on dry ice and stored at -80°C until used. For histopathology, the tissues were fixed in 10% formalin and embedded in paraffin.

Age- and sex-matched (13-14 yrs. male) control human tissues (spleen, lung and brain) were frozen specimens (for lipid analyses) or formalin fixed blocks (immunohistochemistry) from the NICHD Brain and Tissues Bank for Developmental Disorders (University of Maryland, Baltimore, MD). Frozen control human liver samples (0.3-8 yrs.) were from the Cincinnati Children's Hospital Medical Center Biobank.

Antibodies were: anti-CD68 (Ventana, #790-2931), anti-Macrophage mannose receptor (MMR)/CD206 (LifeSpan Biosciences, LS-C150006), rabbit anti-Tau (Phospho-Thr231)-pAb (Gen Script), and anti-Ubiquitin (Abcam, ab7780). The BenchMark XT IHC/ISH Staining Module (Ventana Medical System, Tucson, AZ) was used for immunohistochemistry studies. Tissues sections were counterstained with Hematoxylin.

The paraffin embedded human brain sections were stained with mouse anti-human α -Synuclein and rabbit anti-LC3 as described (7). The tissues sections were deparaffinized and hydrated followed by antigen retrieval in sodium citrate, pH 6.0, using a microwave at power 20% for 10 min. The sections were blocked for 1 hour at room temperature in blocking buffer (5% normal goat serum/0.1 % Triton X-100 in PBS), and then with avidin and biotin, each for 15 min. Primary antibody diluted in blocking buffer was applied and incubated at 4°C overnight. Biotinylated secondary antibody, diluted in blocking buffer (1:1000), was applied for 1 hour after which color was developed with the VECTASTAIN® ABC kit (Vector labs). The tissues sections were counterstained with Hematoxylin.

2.3 Immunoblots

Tissue extracts were prepared as described and protein was detected by immunoblot with mouse anti-human macrophage mannose receptor/CD206, mouse anti-human α -Synuclein, or rabbit anti-LC3 (8).

d. Glycosphingolipid analyses—Glycosphingolipids in frozen tissues samples were extracted and analyzed by TLC (9). Glucosylceramide (GluCer), galactosylceramide (GalCer), glucosylsphingosine (GluS), and galactosylsphingosine (GalS) were quantified by LC-ESI- MS/MS (8).

e. Cholesterol and triglyceride analyses—Tissue cholesterol and triglyceride were extracted and quantified colorimetrically (10).

3. CASE SUMMARY

Earlier findings in this patient were previously reported (3, 5, 6). He was diagnosed with Gaucher disease at 9 months of age following evaluation for hepatosplenomegaly, elevated liver function studies, anemia, and thrombocytopenia. Gaucher disease was confirmed by severely decreased acid β -glucosidase activity in peripheral blood leukocytes and *GBA1* DNA sequencing that showed an allele encoding K79N and an allele with three mutations, L444P, A456P and D409H (Rec2) (3, 5, 6).

ET with mannose-terminated recombinant glucocerebrosidase (imiglucerase [Cerezyme®], Genzyme, a Sanofi Company, Cambridge, MA) was continuous from about 1 year of age, resulting in normalization of hematologic values (hemoglobin, hematocrit, and platelets) by 4.5 years, and significantly decreased liver (1.4 and 1.1 Multiples of Normal (MN)) and spleen (5.7 and 3.4 MN) volumes at 4.5 and 11 years, respectively.

Clinical and radiographic evidence of progressive pulmonary disease was identified at an early age. By 1.5 years of age, the child experienced intermittent episodes of wheezing and dyspnea, which progressed throughout life, to significant respiratory impairment and oxygen requirement at the end of his life. Serial chest CTs, beginning at age 1.5 years, showed progressive, diffuse, patchy ground glass opacities throughout the lungs, consistent with interstitial lung disease, and mediastinal, axillary, and mesenteric lymphadenopathy.

At 3 years of age, abdominal magnetic resonance imaging (MRI) for episodic diarrhea and abdominal bloating demonstrated large nodular, soft tissue masses anteriorly and posteriorly to the renal vein, which, by age 4 years, had enlarged and progressed to surround the aorta, superior mesenteric artery, inferior vena cava, renal vein, and mesentery. The mesenteric masses were palpable. Laparotomy at 5 years revealed extensive lymphadenopathy throughout the mesentery and significant replacement of the normal lymph node architecture on microscopic examination (3). The most recent abdominal MRI at the age of 11 years showed apparent stability of the mesenteric and retroperitoneal lymphadenopathy.

The mesenteric and retroperitoneal lymphadenopathy coincided with progressive malabsorption, protein/electrolyte losing enteropathy, electrolyte dyscrasia, hypoalbuminemia, edema/ascites, poor growth, hypercoagulability/intravascular thrombosis, and osteoporosis. Maintenance of clinical stability required aggressive nutritional intervention, fluid restriction, and electrolyte, and albumin supplementation.

During his 11th year, his disease course accelerated with frequent hospitalizations for management of feeding intolerance (requiring total parenteral nutrition), vomiting and diarrhea, electrolyte disturbances, respiratory disease, and bone fractures.

Global developmental delay, without other overt neurologic signs, was present at diagnosis. By 2 years, saccadic initiation failure and lower extremity hyperreflexia were present. By age 10 years, cerebellar tremor, particularly of the upper extremities, was present at rest and with activity. Progressive ataxia was also noted. Prolonged myoclonus occurred during sleep and generalized tonic-clonic seizures developed. CNS MRIs at 10.5 and at 12.5 yrs showed mildly increased ventricular volumes with enlarged prominence of the cerebral and

cerebellar sulci, and subtle, symmetric, non-specific hyperintense T2/FLAIR signal changes in the thalami. EEG showed a mildly slowed background rhythm with intermittent central spike/slow waves and generalized bursts of high voltage spikes, poly spikes, and slow waves. During this period, his myoclonic and generalized tonic-clonic seizures became intractable to anticonvulsant therapy. The continued progression of his many comorbidities led to his death at 12.5 years.

He continued to receive ET until about 1 month prior to his death. Except for episodic and infrequent febrile illnesses, no evidence was found for concurrent or chronic infectious disease. Neutralizing imiglucerase antibodies (performed by Genzyme) were not detected. Because of the major gastrointestinal problems including protein-losing enteropathy, severe electrolyte imbalances, and nutritional deficits, use of miglustat (Zavesca®, Actelion Pharmaceuticals, Allschwil/Basel, Switzerland) was deemed contraindicated.

4. RESULTS

4.1 Autopsy findings

The liver and spleen were grossly normal with respective weights of 1100 (normal 1010) and 100 (normal 95) gms. In liver a few scattered “Gaucher-like” cells, mild portal fibrosis, and moderate macrovesicular steatosis were present. The fibrosis and steatosis were likely complications of total parenteral nutrition and nutritional/caloric deficits (Fig. 1A).

The spleen was histologically normal. The lungs were heavy, firm, and dense, and weighed 303.5 (left) and 302 (right) gms, (normal, 200 gms). Light microscopy (H&E stain) demonstrated pulmonary scarring and fibrosis as well as extensive alveolar and interstitial storage cells (Fig. 1B). Nodular accumulations of storage cells were identified, particularly around the bronchial airways, pulmonary arteries, and interlobular septae. Abdominal ascites and massive mesenteric lymphadenopathy were present. The cut surface of the lymph nodes revealed a white, chalky substance, which was acellular and eosinophilic (Fig. 1C and Fig. 2).

Intestinal epithelial autolysis precluded detailed evaluation, but there were limited patchy areas of storage cells in the submucosa of the small intestine. The bone marrow was normal. There were no histological abnormalities of the bony trabeculae of the vertebral body and rib, despite the presence of long standing osteoporosis that included the spine. The reason this discrepancy is unclear, but may be related to sampling of individual bones.

Grossly, the brain appeared normal and weighed 1200 gms (normal, 1290 gms). Isolated and nodular accumulations of storage cells were scattered throughout the cerebrum (Fig. 1D). These cells frequently compressed the lumen of arterioles. Similar features were found in the basal ganglia, brain stem, hippocampus, cerebellum, and thalamus demonstrated, particularly surrounding blood vessels (Fig. 3). Cerebral cortical neurons and/or other neurons did not show lipid inclusions. Neuronal loss was prominent in the cerebellar dentate nucleus (Fig. 1E vs. F), but not evident in the cerebral cortex, hippocampus, and basal ganglia. Cerebellar folial degeneration was evident with nearly complete loss of Purkinje cells and severe depletion of the granule cell layer (Fig. 3).

4.2 Immunohistochemistry

The storage cells in the brain stained positively with anti-CD68 antibody, indicating their macrophage lineage (Fig. 4A).

Clusters of CD68 positive microglia/macrophages were found in parenchyma of cerebellum, basal ganglia and hippocampus. Many of them resided in interstitial space of perivascular regions in the brain (Fig. 4A).

The ET products for Gaucher disease, including imiglucerase, contain α -mannose terminated oligosaccharides for preferential targeting to macrophage mannose receptor (MMR) expressing cells (11). MMR signals were detected in the spleen (Fig. 4B), and liver and lung (not shown). In the brain, the MMR signals were detected in a few cells lining small blood vessels (Fig. 4B arrows), but not in the clusters of storage cells around the vessels (Fig 4B, star), and those in brain parenchyma or in any cells within the hippocampus, cerebral or cerebellar cortices or basal ganglia. Using immunoblots, high levels of MMR were detected in extracts from control spleen, and from the patient's liver, lung, and spleen. Such MMR signals were not detected in control hippocampus, or the patient's hippocampus, cerebral cortices, or basal ganglia (Fig. 4C).

Compared to the small and sparse astrocytes, which stained positive with anti-GFAP antibody in the controls, large and engorged GFAP positive astrocytes were present in the hippocampus, basal ganglion and cerebellum from the patient (Fig. 5A a-c) indicating astrogliosis. Many engorged astrocytes surrounded the clusters of CNS macrophages (Fig. 5A b).

Tau hyper-phosphorylation is a marker of neurodegeneration (12). Phosphorylated Tau signals were detected in neuronal soma and processes of the patient's hippocampus, basal ganglion, and cerebellum (Fig. 5A d-f). Most Tau positive cells were in the areas near the clusters of parenchymal storage cells. Accumulation of ubiquitin (Ubi) is a marker for proteasomal dysfunction. Positive Ubi signals were in the patient's white matter, basal ganglia and hippocampus only (Fig. 5A g-i). Aggregations of α -synuclein have been found in Gaucher disease brain from elderly patients and in brains of Gaucher disease mouse models (13, 14). A few cells scattered throughout the patient's cortex and hippocampus had enhanced α -synuclein signals by immunohistochemistry and immunoblots (Fig. 5B a-c and 5C). Increased LC3-II levels are associated with altered autophagic processes. Enhanced LC3 signals by immunohistochemistry and immunoblots were in patient's cortical, hippocampal, and basal ganglia cells (Fig. 5B d and e). TUNEL assays and amyloid precursor protein (APP) staining were negative (data not shown).

4.3 Lipid analyses

GluCer accumulation was in the lymph nodes and lungs (Fig. 6A and Table 1), but not in liver and spleen (Fig. 6A and Table 1). GluS was undetectable in control tissues, only slightly above the detection limit in the patient's liver, spleen and lymph node, but elevated in the lung (Table 1). The fatty acid acyl chains on the GluCer species in lymph nodes and lungs showed similar profiles with GC16:0, GC24:0 and GC24:1 as major accumulated species and with lesser amounts of GluCer 18:0, GC20:0 and GC22:0 (Fig. 6B).

Excesses of GluCer and GluS were in the patient's brain compared to age-matched controls (Table 2). Higher levels of GluCer and GluS were in cerebral cortex, cerebellum, basal ganglia and hippocampus, compared with those in brain stem and spinal cord (Table 2).

These latter areas are myelin-enriched regions with high GalCer contents that were not altered in the patient (Table 2). No GluS was detected in the control brain samples. In the patient, GluS was greater in the cerebellum, basal ganglia and hippocampus, compared to the cerebral cortices. The GluS accumulations correlated with the distribution of macrophage clusters.

GC18:0 GluCer was the major species in the control brain (Fig. 6C). In comparison, various GluCer species, including GC16:0 to GC26:1, were present at increased levels in the patient brain samples. The long fatty acid acyl chain species GC24:1 was predominant in cerebellum, basal ganglia, and hippocampus (Fig. 6C). Overall, the patient brain GluCer species profiles (Fig. 6C) were similar to those in the lung (Fig. 6B), suggesting that the perivascular macrophage storage cells that were on the vascular side of the blood brain barrier contained these longer chain GluCer species and consistent with previous findings in untreated type 2 patients (Table 2) Galactosylsphingosine was detected at equivalent low levels in control and patient brains (data not shown).

Total cholesterol (2.3 mg/mg tissues) or triglycerides (3.1 mg/mg tissues) were not elevated. GCase activity or protein was not detected in patient lymph nodes and liver (data not shown).

5. DISCUSSION

Here, the unanticipated and emerging phenotypic spectrum and limitations in individuals with Gaucher disease who receive ET are highlighted by a 12.5 year-old male who succumbed to type 3 disease. The inability of the ET agent to access the lung, selected lymph node systems, and the vasculature and parenchymal components of the CNS despite 11.5 yrs of therapy highlight the need for additional approaches/strategies to treat these disease aspects. In particular, the apparent unresponsiveness of the CNS perivascular involvement was unexpected and shows the need for further characterization of the nature of the subsets of diseased macrophages in specific tissues.

The mesenteric and mediastinal lymphadenopathy was progressive and causal to a major protein losing enteropathy with attendant comorbidities led to death. In aggregate, these manifestations were life threatening and unresponsiveness to ET. Additional cases of apparently non-progressive lymphadenopathy indicate the non-predictability and severity of this disease complication (18).

Exceptional degrees of histopathological and biochemical variation was observed between organs after 11 years of nearly uninterrupted ET. The expected robust responses to ET were found in the liver, spleen, and bone marrow, even with the presence of a protein losing enteropathy. Conversely, lung, vascular compartments of the CNS, and lymph nodes were severely affected, with pathological features essentially identical to those of untreated Gaucher disease type 3 patients (16). The lack of response to ET in the lung has been

documented at autopsy in some type 2 (19), severe neuronopathic patients, and anecdotal communications suggests a similarly poor response in lymph tissues, including tonsils and adenoids (Grabowski, unpublished observations).

Four types of lung histological findings are present in untreated Gaucher disease: 1) intra-alveolar collections of Gaucher cells, 2) clusters of Gaucher cells in the interstitial space and pleura, 3) periarterial deposits of Gaucher cells, and 4) collections of Gaucher cells in the alveolar capillaries (20, 21). Each of these types existed in the present patient to such a degree that the normal lung architecture was nearly completely obliterated. The source of the lung GluCer is not known, but the alveolar lipid accumulation may arise from tissue endogenous synthesis. This is supported by the normalization of GluCer levels in other visceral tissues with ET, which implies a depletion of distant reservoirs/depots as well as the insufficiency of exogenous enzyme in the alveoli.

The histological findings in the CNS in untreated patients with types 2 and 3 disease include neuronal cell loss in numerous brain structures, including, but not limited to, the basal ganglia, midbrain, cerebellum, hypothalamus, and hippocampus (particularly CA2-4 regions) (13). The storage cells in CNS have shown several types of distributions: (1) Singular to multiple parenchymal Gaucher cells (particularly in cerebral cortex and cerebellum); (2) storage cell accumulations in perivascular spaces (particularly in the cerebral cortex); and (3) a combination of the two (16, 20-22). Pattern 1 appears more commonly present in the younger patients with neuronopathic disease (20), whereas the perivascular and the periadventitial pattern is more common in older individuals with neuronopathic disease or much older patients with type 1 disease (20, 23). This suggests that over time, the storage cells migrate to and remain in the perivascular spaces (20, 21). A long-standing hypothesis has been proposed, but not tested with modern imaging technology, that the brain pathology/damage is associated with changes in blood flow/ischemia due to the globally distributed perivascular lesions encroaching on the vascular lumen (24). Hippocampal pyramidal neuron cell loss was reported in Gaucher disease type 2 patient brain (23). Neuron loss was prominent in the cerebellar dentate nucleus and Purkinje cell layer, but not remarkable in hippocampus and basal ganglion in this patient. Hypoxia is a leading cause for hippocampal neuron loss. The patient brains from these studies could be under a different degree of hypoxia that resulted variation on hippocampal neuron loss. Finally, loss of Purkinje cells and subsequent ataxia, as seen in our patient, has been documented in untreated patients (13). Importantly, all these findings on either side of the blood brain barrier are nearly identical to those previously reported in patients who have not received ET (20, 21).

The clusters of CD68 positive macrophages/microglia were distributed in broad regions throughout the patient's brain. However, their origin(s) is not clear due to the lack of specific markers for endogenous microglia. The two populations of CD68 positive microglia cells included endogenous microglia that derive from yolk sac stem cells, are resident in brain parenchyma permanently, and when activated assume an amoeboid shape. In comparison, perivascular microglial cells are derived from bone marrow precursors by myeloid differentiation and continuously turned over (25). These latter microglia have functions related to vascular wall repair (26). In addition to microglia, monocyte-derived

perivascular macrophages reside in interstitial space in brain perivascular areas (27). Those macrophages likely originate in the viscera and are the main phagocytes for lipid accumulation (15). The GluCer species profile in the CNS from untreated patients with Gaucher disease was similar to that from visceral organs.

Surprisingly, despite 11 years of ET and the normalization of GluCer contents in liver, spleen, and bone marrow, the GluCer species profile in the CNS of the present patient still retained the visceral signature. This was unexpected because of the normalization of GluCer in the visceral sources, and the perivascular macrophages would have been thought to be capable of taking up the administered mannose-terminated GCse.

Recombinant GCses for ETs have terminal α -mannosyl residues for targeting to the MMR for preferential uptake into macrophages (28), with additional uptake into endothelial cells and hepatocytes (29, 30). However, intravenous (IV) ET has little penetration into brain parenchyma because of the selective permeability of the blood brain barrier. As a result, the CNS *per se* would not experience major and/or direct positive effects of IV ET. In a short-term study, this lack of effect was shown in the rapidly progressive CNS disease in Gaucher disease type 2 (31). The extensive brain pathology in the present patient clearly shows that even long-term ET has essentially no effect on the parenchymal CNS involvement by neuronopathic Gaucher disease (type 3). The lack of ET effect on the perivascular and periadventitial accumulations of Gaucher cells was unexpected. Importantly, the MMR is expressed on neural cells, including Schwann cells, astrocytes, and neurons (32, 33) that are developmentally regulated with neonatal expression being higher than that in the adult brain (34). Also, MMR is normally present on perivascular macrophages/microglia and meningeal macrophages, but not on parenchymal microglia in normal brain (34). MMR was not detected in the present patient's brain parenchyma cells, and importantly, in only scant cells lining blood vessels. Indeed, MMRs were not detected on CD68 positive storage cells lining the perivascular space or in the parenchyma. MMR expression is up regulated in alternatively activated/M2 macrophages and this can be enhanced by IL4 and inhibited by IFN γ (35, 36). The absence of MMR in brain storage cells, but presence in such visceral cells (i.e., not a deficiency state in this patient) indicates that these macrophages are similar to those in the Gaucher patient's spleen in which majority macrophages are M2 macrophages (37). The presence of MMR on some lung storage cells suggests that the lack of MMR expression/detection on storage cells in the CNS of the present patient and untreated Gaucher disease spleen (37) is not due only to the accumulated GluCer or GluS, but other factors determine this tissues specificity. This was suggested by the absence of MMR in age matched control CNS samples. Importantly, these data also indicate the inability to rid the CNS of perivascular storage cells. The potential contribution of these storage cells to the Gaucher disease process in the CNS represents another compartment that is poorly accessible to ET.

Consistent with histological findings, high levels of GluCer and GluS in the lung, lymph node, and brain were not corrected by IV ET. Our findings were comparable to the data from untreated patients with Gaucher disease type 3 (Tables 1 and 2). Although the quantification methods are different (gas chromatography vs. LC/MS), the GluCer and GluS levels are in the same range. GluCer and GluS levels were greatest in gray matter, basal

ganglia, and hippocampus. Whereas, in white matter enriched regions, i.e., brainstem and spinal cord, GluCer and GluS levels were very low. This may reflect cell type (neuron and microglia vs. oligodendrocyte) specific GluCer metabolism. The predominance of longer chain fatty acid acyl chains on GluCer (i.e., the visceral pattern) in the brain indicates that the perivascular storage macrophages are most likely the major repository of these lipids in the brain. The extracerebral origin of such long acyl chain GluCers in Gaucher disease type 3 was originally suggested by Nilsson and Svennerholm (15).

Enhanced Tau, α -synuclein, and ubiquitin signals in the patient brain indicated a more generalized protein degradation defect as has been described in more common neurodegenerative diseases, e.g. Parkinson disease and Alzheimer disease (12). Phosphorylated Tau and total Tau were detected, but in a normal range, in CSF of a Gaucher disease type 1 patient who was also diagnosed with Parkinson disease. The patient had the following *GBA1* mutations: compound heterozygosity for [c.115+1G>A and c.1226A>G (IVS2 + 1/N370S)] (38). The present patient showed aberrant hyperphosphorylation of tau. Although excess α -synuclein and ubiquitin were observed in the current patient, aggregations of these proteins were not whereas they are in the more common synucleinopathies (14, 39, 40). The patient's age (12.5 years) may have not allowed sufficient time for aggregations to form. Increased ubiquitin and LC3 signals support defective protein degradation via the proteasome and autophagy pathways, reflecting generalized neurodegeneration, which could have resulted from defective lysosomal homeostasis caused by substrate accumulation and/or inflammatory insult from those Gaucher cells.

Alternative approaches are needed for treating these restricted regions, e.g., brain and lung disease, in Gaucher disease. The recent FDA approval of eliglustat (41), a glucosyl ceramide synthase inhibitor, may provide such an approach for visceral, but not parenchymal CNS, sequestered compartments as was shown for lungs in a Gaucher disease mouse model (42). Understanding the nature and function of brain microglia and perivascular macrophages, and lung macrophages in the Gaucher disease will facilitate the development of improved therapeutic approaches.

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Abbreviations

APP	Amyloid precursor protein
CNS	Central nervous system
ET	Enzyme Therapy

IV	Intravenous
GCase	glucocerebrosidase
GluCer	glucosylceramide
GluS	glucosylsphingosine
GFAP	Glial Fibrillary Acidic Protein
MMR	macrophage mannose receptor
Ubi	ubiquitin

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Highlights

- Significant histological variation existed between organs in the patient.
- Specific tissues/organs (brain, lung & lymph node) had no response to ET.
- Neurodegeneration and proinflammation were in the brain despite long term ET.
- No MMR expression was detected on storage cells in the CNS of the patient.
- The GluCer species in the CNS of the patient had the visceral signature.

Figure 1. Pathological findings by H&E

A) Liver histology: Moderate steatosis (arrow) and mild portal fibrosis (arrow ahead) were noted. B) Lung histology: Extensive accumulation of storage cells in alveolar spaces (arrow). C) Mesenteric lymph node histology: The lymphoid architecture was significantly replaced with an acellular material with areas of calcification. D) Pathology of cerebrum: The storage cells (arrow) consisted of large histiocytes with vacuolated cytoplasm and containing typical inclusions for Gaucher disease frequently compressed the lumen of the arterioles. E) Decreased neurons (arrow) in patient cerebellar dentate nucleus. F) Neurons (arrows) in unaffected age matched human control cerebellar dentate nucleus.

Figure 2. Mesenteric lymph node

Cut section of Mesenteric lymph node showing replacement of gross structure with a white, chalky material.

Figure 3. H&E staining of paraffin brain sections

The storage cells (arrows) were in parenchyma of cerebellum and hippocampus and lining around perivascular region in basal ganglia from the patient. Normal Purkinje cell (arrowheads) layer was in control cerebellum. Loss of Purkinje cells (arrowheads) was prominent in patient cerebellum.

Figure 4. Storage cells and Macrophage mannose receptor (MMR) expression

(A) The storage cells were positive for CD68 antibody staining (brown), a macrophage marker, and were present in patient's cerebellum, hippocampus, basal ganglia. (B) MMR positive staining (brown) was in patient spleen. A few MMR positive cells (arrows) were around vessels in the basal ganglia and hippocampus. The storage cells (star) in the hippocampus did not stain positive for MMR. The nuclei were counter stained with hematoxylin (blue). Scales are indicated in the images. (C) Immunoblot of MMR. Control and patient spleen (Sp), liver (Li) and lung (Lu) expressed MMR protein. Control and patient brain samples had no detectable MMR protein. Hi, hippocampus; Ba, basal ganglia; Co, cerebral cortex. β-actin was used as loading control.

Figure 5. Brain pathology

(A) Astrogliosis, Tau and Ubiquitin (Ubi) pathology. (a-c) Astrogliosis (brown) was determined by anti-GFAP staining. The control human basal ganglia (a) had low level GFAP signal. The engorged astrocytes (arrows) were detected in patient hippocampus (b) and cerebellum (c). (d-f) Tau pathology was assessed by anti-phospho-Tau antibody. No phospho-Tau signal was detected in control human basal ganglia (d). Strong phospho-Tau signals (brown) were in the cells (arrows) of patient basal ganglia (e) and hippocampus (f). (g-i) Ubi signals (brown) were detected in white matter areas of patient basal ganglia (h) and around storage cells (arrowhead) in hippocampus (i). No positive Ubi staining was observed in basal ganglia white matter in control (g). (B) α -Synuclein (α -SYN) and LC3 detection. Low level of α -SYN signals were detected in the cells of control human hippocampus (a). Enhanced α -SYN signals were found in a few cells in the patient's cerebral cortex (b) and hippocampus (c). In comparison to control hippocampus (d), LC3 signals were shown in patient's hippocampus (e). Scales are indicated in the images. Nuclei were counter stained with hematoxylin. C. Immunoblots. Left, α -SYN levels were increased in patient hippocampus (Hi) and cortex (Co) compared to control hippocampus. Right, Increased LC3-II was detected in patient hippocampus (Hi) and cortex (Co). β -actin was used as a loading control.

Figure 6. Glycosphingolipid analyses

(A) TLC analyses of lymph node and liver. Patient lymph node had accumulated glucosylceramide (GluCer, GC). GC in patient liver was at control (normal) level. Lipids standards (std) on TLC: GC, glucosylceramide; Sulf, sulfatide; and SM, sphingomyelin. (B) GluCer species profile in visceral tissues. Patient lung and lymph node had accumulation of short and long chain GluCer species compared to control lung. (C) GluCer species profile in patient CNS. GC18:0 is the major species in control brains (black bars). In addition to GC18:0, GC16:0 and various long fatty acid acyl chain GluCer species were detected and increased in patient cortex, cerebellum, hippocampus, basal ganglion, brain stem and spinal cord as shown in colored bars. More GluCer species were accumulated in cortex, cerebellum, hippocampus, basal ganglion than that in brain stem and spinal cord. GluCer species were analyzed by LC/MS and normalized by mg of tissue weight.

Table 1

Glycosphingolipids in viscera of the Gaucher disease type 3 patient and controls

		GluS	GluCer
		(pmol/mg tissue)	
Tissues			
Present Patient			
12.5 yrs	Liver	0.9	11.1
	Spleen	1.5	41.0
	Lung	183.8	1321.6
	Lymph node	2.8	382.9
Controls			
0.3-8 yrs	Liver (n=4)	ND	21.3
13 yrs	Spleen (n=2)	ND	29.3
13 yrs	Lung (n=2)	ND	8.7
11 yrs [*]	Liver	ND	34.0

ND, Not detectable.

*
Data from reference (15)

Table 2

Glycosphingolipids in the CNS of Gaucher disease type 3 patients and controls

		GluS	GluCer	GalCer
	Tissue	(pmol/mg tissue)		
Present Patient				
12.5 yrs	Cerebral cortex	1.7	27.1	402.3
	Cerebellum	10.2	319.1	328.4
	Hippocampus	3.0	263.2	249.0
	Basal ganglion	6.9	475.7	600.7
	Brain stem	0.7	4.5	1288.8
	Spinal cord	0.4	5.8	1502.6
Control				
13 yr	Cerebellum	ND	1.1	597.4
	Hippocampus	ND	0.8	838.1
	Basal ganglion	ND	1.8	982.1
Data from references				
GD Patients (15)				
2-12 yr	Cerebral cortex (n=8)	0.8-4.6	37-65	750-2330
	Cerebellar cortex (n=6)	1.4-6.3	59-1750	2800-5940
Controls (15-17)				
6-24 mos	Cerebral cortex (n=11)	ND	5.0	980.0
5-8 mos	Cerebellar cortex	ND	10.0	1610.0
5-20 yr	Cerebellar cortex (n=9)	ND	16.0	6030.0

ND, Not detectable