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Original article

Early initiation of ambroxol treatment diminishes neurological manifestations of type 3 Gaucher disease: A long-term outcome of two siblings

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ABSTRACT

Gaucher disease type 3 (GD3) is a severely debilitating disorder characterized by multisystemic manifestations and neurodegeneration. Enzyme replacement therapy alleviates visceral signs and symptoms but has no effect on neurological features. Ambroxol has been suggested as an enzyme enhancement agent. Some studies have confirmed its effectiveness in preventing the progression of neurological manifestations of neuronopathic Gaucher disease. In this study, we report two GD3 siblings in whom ambroxol combined with enzyme replacement therapy was initiated at different stages of the disease. We demonstrate the enzyme enhancement effect of ambroxol on L444P/H225Q;D409H glucocerebrosidase activity through results of fibroblast studies and long-term clinical outcomes of the two patients. The sibling diagnosed at the age of four-and-a-half years with significant neurological involvement manifested relatively rapid improvement on ambroxol treatment, followed by stabilization of further course. The younger sibling, in whom the treatment was started at seven weeks, displayed attention deficit and low average cognitive functioning at the age of seven years, but did not manifest other neurological symptoms. The difference in neurological outcomes indicates that ambroxol delayed or even halted the evolution of neurological manifestations in the younger sibling. This observation suggests that early initiation of ambroxol treatment may arrest neurological involvement in some GD3 patients.

1. Introduction

Gaucher disease (GD) is an inherited lysosomal storage disorder caused by insufficient activity of enzyme beta-glucocerebrosidase (glucosylceramidase, EC 3.2.1.45) (GCase) due to biallelic mutations in the *GBA* gene. As a consequence of enzyme deficiency and disturbed breakdown, glucocerebrosides are accumulated in lysosomes of the monocyte-macrophage system of various tissues; primarily the liver, spleen, bone marrow, lymph nodes, but also in the central nervous system (CNS). GD is a multisystemic, progressive, and debilitating disease characterized by hepatosplenomegaly,

Abbreviations: ABX, ambroxol; ADHD, attention deficit and hyperactivity disorder; BBB, blood-brain barrier; CBC, complete blood count; CHT, chitotriosidase; CNS, central nervous system; CSF, cerebrospinal fluid; DEXA, dual-energy X-ray absorptiometry; eow, every other week; EET, enzyme enhancement therapy; ERT, enzyme replacement therapy; GCase, beta-glucocerebrosidase; GD, Gaucher disease; HSCT, hematopoietic stem cell transplantation; Lyso-Gb1, glucosylsphingosine; VEP, visual evoked potentials; VPA, valproate.

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cytopenia, bone marrow infiltration, pulmonary disease, skeletal involvement, and predisposition to autoimmune disease and malignancy [1]. Although it has lately been considered a phenotypic continuum [2], GD is traditionally classified into three types according to the clinical course and neurological involvement. Type 1 (GD1, OMIM 230800) is the most common form, known as the non-neuropathic form. Patients have a multisystemic disorder, but don't manifest the primary neurological features. Type 2 (GD2, OMIM 230900) is an acute neuronopathic form characterized by early and rapidly progressive neurodegeneration and death in infancy or early childhood. Type 3 (GD3, OMIM 231000) is a chronic neuronopathic form with slowly progressive neurological deterioration.

About half of the patients with neuronopathic GD manifest their first neurological symptoms by the age of two years [3]. Some patients are difficult to distinguish clinically and classify into type GD2 or GD3 so an intermediate phenotype of neuronopathic GD had been suggested [4]. Neurological symptoms in GD3 represent a clinical spectrum manifesting in infancy, childhood, or rarely in adulthood. Common neurological features are oculomotor apraxia, strabismus, seizures, progressive myoclonic epilepsy, ataxia, developmental delay, learning disability, and dementia [5].

Common mutation in GD3 patients is the c.1448T>C (p.L483P), usually reported in literature using alternate nomenclature L444P, which is present in homozygosity in up to 70% of patients [6]. Patients harboring the same genotype may manifest different courses of the disease and therefore other genetic and/or environmental factors may contribute to the phenotype heterogeneity [7]. For instance, monozygotic twins with a highly discordant phenotype, one twin with visceral and neurological involvement consistent with GD3, and the other without any signs or symptoms of GD, were reported [8].

Current recommendations for treatment of patients with GD3 include enzyme replacement therapy (ERT) which has a very good safety profile, ameliorates visceral involvement and hematological abnormalities [9]. Gaucher registry data confirmed the efficacy of ERT for treating non-neurological symptoms and demonstrated that current recommendations for systemic management of GD3 seem to be appropriate. Unfortunately, there is no evidence that ERT has any effect on neurological symptoms [6]. There were reports about the positive effect of hematopoietic stem cell transplantation (HSCT) on stabilizing or even correcting neurological changes in GD3 patients, but there was not enough long-term evidence to support this therapeutic approach [10]. On the contrary, long-term follow-up of two patients showed that HSCT had no effect in preventing the development of neurological symptoms [11]. Experiences from a few other centers also showed that HSCT failed to rescue neurological phenotype in GD3 patients (personal communication). Substrate reduction therapy with miglustat, a small molecule drug that crosses the blood-brain barrier (BBB), failed to show significant benefits on neurological manifestations [12]. A more potent, highly-specific ceramide-mimetic inhibitor, eliglustat, is also ineffective in preventing neurological disease, as this molecule is recognized by multidrug resistance protein MDR1 and does not cross the BBB [13]. Enzyme enhancement therapy (EET) or pharmacological chaperones are small molecules that easily cross the BBB, bind to the mutant protein enabling its correct folding thereby stabilizing the protein and enhancing enzyme activity. Ambroxol (ABX) has been identified as a pharmacological chaperone capable of folding the defective GCase correctly. ABX is a pH-dependent, mixed inhibitor of GCase, whose inhibitory activity is maximal at neutral pH present in the endoplasmatic reticulum, and undetectable at acidic pH of lysosomes. ABX was shown to function as an EET agent in GD1 and GD2/3 patient-derived cultured skin fibroblasts [14]. ABX hydrochloride is a Na^+ channel blocker widely used as a commercially available expectorant and previously used in respiratory distress syndrome with long experience of clinical use and no serious side effects. Murine experiments showed that oral ABX had good bioavailability with distribution and enzyme enhancement activity in various tissues, including the brain, and with lack of acute toxicity [15]. ABX given at 25 mg/kg per day to a maximum daily dose of 1300 mg in a study of five GD3 patients had a good safety and tolerability profile and resulted in increased lymphocyte GCase activity and decreased glucosylsphingosine (Lyso-Gb1) in cerebrospinal fluid (CSF). All patients manifested improvement of neurological symptoms, including reduction of myoclonus, seizures, and pupillary light reflex dysfunction [16].

In this paper, we present the long-term effects of ABX treatment on neurological features in two siblings with GD3. Both parents signed informed consent form before ABX therapy was started and the off-label treatment was approved by the Local Drug Committee.

2. Patients, materials and methods

2.1. Patients

Here we delineate patient characteristics before ABX treatment. Clinical effect of ABX treatment is described in the section Results.

2.1.1. Sibling 1

Sibling 1 is the first, female child of healthy and unrelated parents. In the newborn period, tricuspid atresia type 1B was diagnosed and balloon atrial septostomy had been performed. At the age of three months, she had a partial cavopulmonary shunt. Total correction, a total cavopulmonary anastomosis, was done at the age of two years and three months. At this point, hepatosplenomegaly was noted (the liver and spleen were palpable 4 cm below the costal margins). Convergent concomitant strabismus developed around the age of two years. At the age of three, a massive splenomegaly was discovered during a regular cardiology assessment. An abdominal ultrasound revealed a normal liver and enlarged spleen (spleen size 14×6 cm; for children aged four to six years the mean spleen length is 7.9 cm, 95th centile 9.4 cm [17]). Complete blood count, electrolytes, BUN, creatinine, aminotransferases, total proteins, protein electrophoresis, immunoelectrophoresis, and peripheral blood smear were normal. Epstein-Barr virus, cytomegalovirus, adenovirus, and toxoplasma serology tests were negative. Technetium-99 M sulfur colloid scintigraphy showed no focal changes in the massive spleen. At the age of four years and nine months, the child experienced a generalized seizure for the first time. EEG showed multifocal discharges. At this point, she had delayed growth (BW and BL Z score −2), ataxia, stridor, dysarthria, convergent strabismus, horizontal gaze palsy, oculomotor apraxia, and hepatosplenomegaly. The liver size was 10×15 cm (for children aged four to six years the mean longitudinal liver length is 9.12 cm, 95th centile 10.91 cm) [18], and the spleen 16×6 cm, measured on ultrasound. Brain MRI revealed mild brain stem and cerebellar atrophy. Brainstem auditory evoked response was normal while the visual evoked potential (VEP) was abnormal, showing decreased post-retinal visual activity – low binocular and monocular onset and reversal amplitudes. Psychological assessment showed below-average results, with a delay in gross and fine motor development (Z score −2.4). Lumbar spine dual-energy X-ray absorptiometry (DEXA) revealed osteoporosis (Z score −3.6). Laboratory findings showed mild thrombocytopenia (thrombocytes 119×10^9/L, reference range 150-450), and mildly elevated aminotransferases - ALT 65 U/L (reference range 24-49), AST 28 U/L (reference range 9-20). Diagnosis of GD was confirmed by measuring low GCase activity in leukocytes (0.9 nmol/mg protein/h, reference range 5.4-11.6) and very high chitotriosidase (CHT)

activity in the serum (>12 000 mU/mL; reference range 0-200). *GBA* gene analysis revealed three pathogenic mutations, c.1448T>C (p.L483P) and c.882T>G/1342G>C (p.H294Q/D448H) on the other allele, alternate nomenclature H225Q;D409H. ERT with imiglucerase (Cerezyme, Sanofi Genzyme) was started at the dose of 60 IU/kg every other week (eow). Three months after the first seizure, she had an episode of unresponsiveness and bilateral spike-and-wave discharges on EEG, hence valproate (VPA) treatment was started. Two weeks later, at the age of five years (two months after ERT initiation), peroral ABX treatment was started at 25 mg/kg/day in five divided doses.

2.1.2. Sibling 2

At the time the diagnosis was confirmed in the older sibling, the mother was in the third trimester of pregnancy; therefore, prenatal testing was not done. Sibling 2, a male, was diagnosed at the age of two weeks by enzyme testing (GCase in leukocytes 0.67 nmol/mg protein/h), and confirming the same *GBA* genotype as was found in the older sibling. The first clinical evaluation, at six weeks, showed the boy had mild splenomegaly (8.3×5.5 cm on sonography; for infants up to three months mean spleen length is 4.6 cm, 95th centile 6.1 cm [17]) and mild thrombocytopenia (thrombocytes 135×10^9/L). Peroral ABX treatment, 25 mg/kg/day divided into six doses, was started at the age of seven weeks. ERT with imiglucerase was commenced at the age of three months, at 60 mg/kg eow. At the time of ERT initiation the spleen was still mildly enlarged, but thrombocytopenia had already resolved, and the patient had no other clinical signs of the disease.

Before starting a long-term ABX treatment, we tested the ABX enhancing effect on mutant GCase in patient-derived fibroblasts.

2.2. In vitro test of glucocerebrosidase enhancement by ambroxol

Isolation and cultivation of human skin fibroblasts was performed according to the standard operating procedures corresponding to the EuroBioBank protocols [19]. To evaluate the enzyme-enhancing effect of ABX on GCase in patient-derived dermal fibroblasts, a fibroblast culture was grown and separated in the media without ABX, and with different ABX concentrations, i.e. 1, 5, and 10 μM, for five days. We determined ABX concentrations in the media according to the results of our pilot assay on patients' fibroblasts and previously published results showing normal viability of both wild type and GD patient-derived human fibroblast cultures at ABX concentrations up to 30 μM, and cytotoxic effect at higher concentrations [15]. ABX media was enriched with Ambroxolhydrochlorid (Euro OTC Pharma GmBH, Germany). Culture media was replaced after 60 h with fresh media without ABX or supplemented with ABX at the indicated concentrations. Subsequently, the media was removed, and the cells were washed twice with phosphate-buffered saline (Ca^2- and Mg^2-free), and harvested by trypsinization. Following centrifugation, cells were lysed and left on ice for 30 min before enzyme analysis.

2.3. Glucocerebrosidase enzyme activity in fibroblasts and leukocytes

The assay to measure GCase enzyme activity in fibroblasts and leukocytes was carried out with an in-house method using 4-methylumbelliferyl-β-D-glucopyranoside as an artificial substrate [20]. Reference range for GCase in leukocytes is 5.4–11.6 nmol/mg protein/h, and in fibroblasts 85–373 nmol/mg protein/h.

With the aim to test the effect of ABX on GCase activity *in vivo*, we compared the baseline GCase activity in leukocytes to activity during ABX treatment. As patients also regularly received ERT, which may increase enzyme activity in leukocytes following dose application to normal values, we measured the activity in leukocytes just before ERT infusion.

2.4. Biomarker analysis

CHT activity was measured in serum and the CSF with 4-methylumbelliferyl-β-D-N,N′,N″-triacetylchitotriose according to a previously published method by Hollak and co-authors [21]. Reference range for CHT in serum is 0-200 mU/mL, and in the CSF <8 mU/ml.

Glucosylsphingosine (Lyso-Gb1) was measured using high-pressure liquid chromatography - tandem mass spectrometry in Centogene AG, Rostock, Germany [22]. Lyso-Gb1 reference range in serum is <1.0 ng/mL.

3. Results

3.1. Effect of ambroxol on glucocerebrosidase activity in fibroblasts

Enzyme assays in fibroblast cultures derived from both patients following a 5-day incubation period with different concentrations of ABX in the cell media (0, 1, 5, 10 μM) showed the maximum GCase enzyme enhancement effect at ABX concentration of 10 μM (Fig. 1).

3.2. Effect of ambroxol on glucocerebrosidase activity in leukocytes

GCase activity in leukocytes from blood samples drawn just before the next ERT infusion and during ABX treatment were significantly higher than the baseline GCase activitiy in both patients (Tables 1 and 2). The increase of GCase activitiy in leukocytes during ABX treatment might reflect the enzyme enhancement effect of ABX.

3.3. Biomarkers in serum and cerebrospinal fluid before and during enzyme replacement therapy and ambroxol treatment

3.3.1. Chitotriosidase

At diagnosis, sibling 1 had markedly increased CHT activity in the serum, more than 214-fold of the control (>12 000 mU/mL, control 56). A year after treatment was started, CHT normalized and remained within the reference range during the follow-up period.

Sibling 2 had increased CHT activity at diagnosis, and at two weeks of life it was 27-fold higher than the control (1534 mU/mL, control 56). CHT normalized three months after the initiation of treatment and remained within the reference range during the follow-up period.

CHT activity was measured in the CSF before and after 3, 6, and 9 months of ABX treatment (with the exception of CHT activity in the CSF of sibling 1 after six months on ABX, which was not possible due to a dry tap). CHT activity in the CSF initially decreased in both patients after three months of ABX treatment, but subsequent analyses didn't show a similar trend (Fig. 2). Additional measurements were not possible as parents refused lumbar punctures.

3.3.2. Glucosylsphingosine

At diagnosis, serum concentration of Lyso-Gb1 in sibling 1 was 418 ng/mL (reference range <1.0 ng/mL), after two months of ERT and before ABX treatment it was 214, and after three months of combined ERT and ABX treatment it decreased to 44.9 ng/mL. Serum concentration of Lyso-Gb1 in sibling 2 was 46 before ABX and ERT treatment, and after three months of combined treatment it decreased to 11 ng/ml. Regarding the concentrations in CSF, sufficient samples for analysis were attainable only for sibling 1 whose Lyso-GB1 concentration in CSF before ABX treatment was

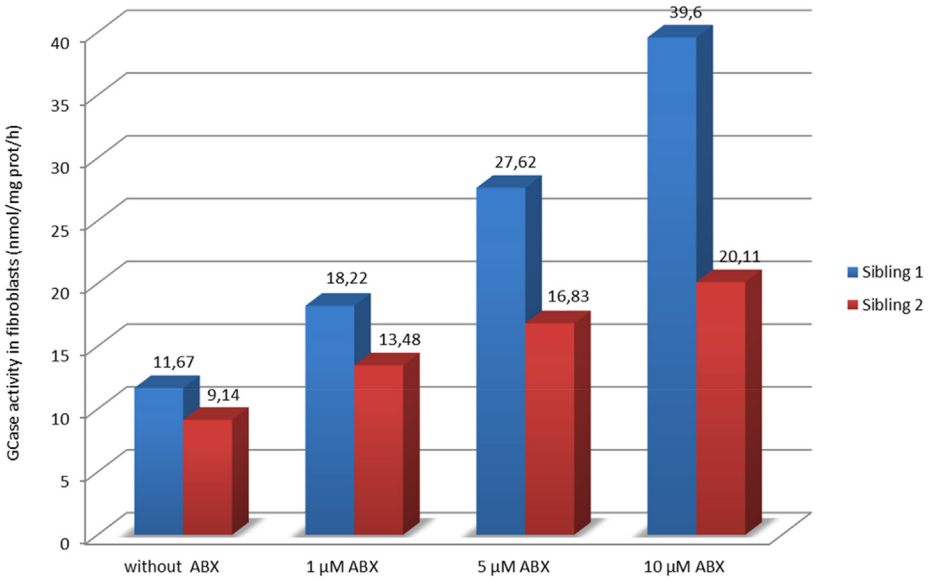

Fig. 1. Enzyme enhancement effect of the different ABX concentrations on mutant GCase activity in fibroblast culture. Enzyme activities are presented graphically in absolute values. Incubation with 1, 5, and 10 μM ABX in culture media resulted in GCase enhancement of 56%, 136.7%, and 239.3%, respectively, in fibroblasts of sibling 1 (blue bars), and 47.5%, 84%, and 120%, respectively, in fibroblasts of sibling 2 (red bars). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Activity of glucocerebrosidase in leukocytes ERT and ABX treatment in sibling 1.

Before the treatment (at the time of diagnosis)		0.99 nmol/mg protein/h
On ABX treatment	Before ERT dose	1.32 nmol/mg protein/h
	2 h after ERT application	5.44 nmol/mg protein/h

Table 2
Activity of glucocerebrosidase in leukocytes before therapy and on ERT and ABX treatment in sibling 2.

Before the treatment (at the time of diagnosis)		0.48 nmol/mg protein/h
On ABX treatment	Before ERT dose	1.45 nmol/mg protein/h
	2 h after ERT application	6.20 nmol/mg protein/h

0.5 ng/mL, and after three months of ABX treatment decreased to 0.1 ng/mL.

3.4. Clinical outcomes of long-term ambroxol treatment

3.4.1. Outcome of sibling 1

At the first evaluation, three months after initiating ABX treatment, the parents reported sibling 1 had better stamina, with less expressed oculomotor apraxia, dysarthria, and stridor. In the following period, there were no significant changes in the neurological status. In parallel, there was resolution of all visceral signs of GD and laboratory abnormalities (thrombocytopenia, increased CHT activity) within a year after ERT was started. Control VEP, performed 3, 9, and 21 months after ABX treatment started, showed

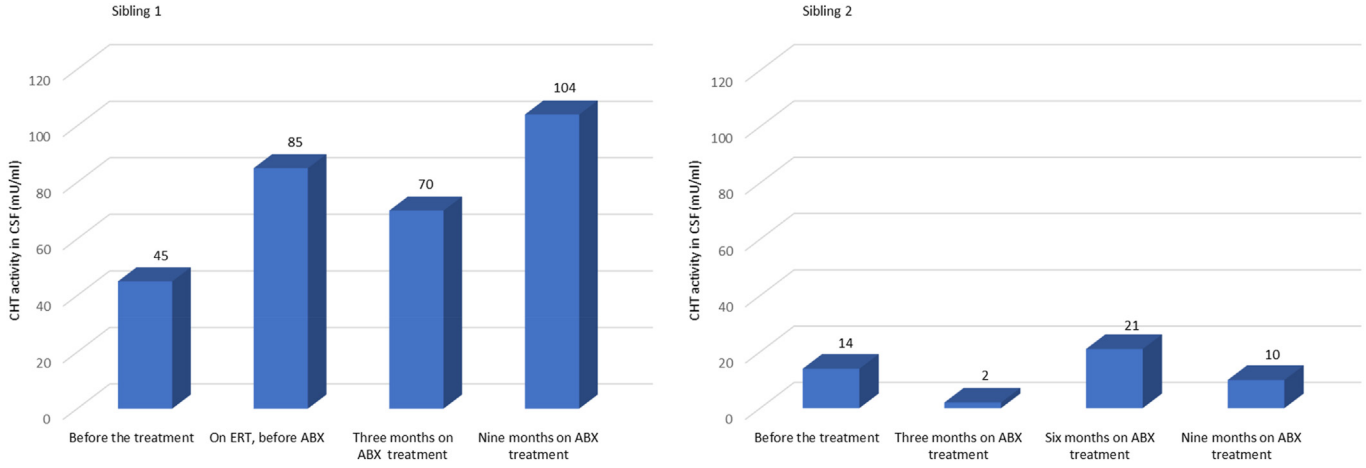

Fig. 2. CHT activity in the CSF of two siblings during ABX treatment. The results of sibling 1, presented on the left graph, show that CHT activity slightly decreased after three months, but increased again after nine months of ABX treatment (at the same time serum CHT activity was within the reference range). The results of sibling 2, presented on the right graph, show that CHT activity normalized after three months, increased again after six months, and after nine months of ABX treatment declined to the value lower than the baseline. Reference range for CHT in the CSF is <8 mU/ml.

better response reproducibility and higher amplitudes (Supplementary material). The patient was seizure-free since ABX treatment started. The dose of VPA was adjusted according to the weight gain to achieve therapeutic blood concentration. Follow-up EEGs showed dysrhythmia. The parents refused a follow-up brain MRI under anesthesia. At the last evaluation, at twelve years, the patient did not have physical disability that would interfere with everyday activities and attended regular school by an individualized education program.

3.4.2. Outcome of sibling 2

Visceral signs resolved after three months of ABX and ERT treatment. In the follow-up period, and at the last evaluation, at age seven years, the patient did not have signs or symptoms of the disease except for low average/borderline cognitive functioning, with a linear trend, and therefore not necessarily attributable to GD3.

3.4.3. Psychological assessment of sibling 1

At diagnosis, the patient's overall development was in the below-average range (Z score −1.3), with uneven development of different cognitive functions: the speech was in the average range and fine and gross motor functions were mildly delayed. At subsequent psychological evaluations (at age 6, 7, 8, 9, 10, and 11 years), multiple cognitive deficits were more evident (deficits in learning, memory, attention, processing speed, visuospatial reasoning and perceptual organizational skills), which contributed to stagnation/slowdown in cognitive development although the patient didn't lose previously learned skills or manifest any sign of cognitive deterioration. The patient's verbal reasoning ability remained in the borderline/low average range (Z score −1.3), but due to the previously mentioned multiple cognitive deficits that interfered with learning and cognitive development, overall cognitive functioning was between mild intellectual disability and borderline intellectual functioning (Z score −2.1).

3.4.4. Pshychological assessment of sibling 2

At the baseline psychological assessment, at eleven months, the patient's development was in the below-average range (Z score −1,3) with attention deficit noted. At subsequent psychological evaluations (at 2, 3, 4, 5, and 6 years), there was no declining or stagnation in cognitive development and even a mild improvement in attention occurred, even though the patient had attention deficit and hyperactivity disorder (ADHD). At the age of three and a half years, he was included in speech and language developmental therapy. Psychological evaluation at the age of six years showed that overall cognitive functioning was in the low average/borderline range (Z score −1.3). The test profile indicated that his cognitive potential was in the average range (in the area of nonverbal reasoning), while verbal reasoning and visual-spatial functions were in the low average/borderline range. Attention deficit difficulties had a negative impact on cognitive efficacy. It is worth emphasizing that detailed psychological testing could not be performed due to ADHD and lack of cooperation.

When compared with sibling 1, sibling 2 had more linear development, without stagnation or slowdown. Besides ADHD, no other prominent cognitive deficit was noted. Also, when comparing both siblings at age six years, sibling 2 had better developmental scores in all areas, except verbal reasoning, and overall cognitive functioning was significantly better.

3.4.5. Safety assessment

During the first months of ABX treatment the parents reported increased mucus production and soft stools in both children which didn't cause significant problems. We didn't observe any clinical side effects and follow-up routine laboratory findings remained within the reference range with the exception of mildly prolonged prothrombin index (0.68 and 0.66, normal >0.7) in sibling 1, after five and six years of ABX treatment, which later spontaneously normalized.

4. Discussion

In this study we demonstrated a positive effect of ABX on GCase activity in fibroblasts and leukocytes, and on the outcome of two siblings, compound heterozygotes for common L444P mutation and double mutant allele H225Q;D409H. The double mutant allele is characteristic for the Balkan area. Previous results of *in vitro* expression studies have shown that concomitant presence in *cis* of H255Q and D409H mutations practically abrogates the enzyme activity. These findings were in accordance with observed phenotype as patients homozygous for H225Q;D409H allele manifested GD2 [23]. L444P/H225Q;D409H genotype had been previously reported in GD3 patients. The aim of our study was to investigate the effect of ABX on GCase activity and clinical outcome in patients with L444P/H225Q;D409H genotype. Results of earlier studies indicated that fibroblasts derived from GD patients containing homozygous L444P mutation didn't manifest a significant increase of GCase activity in the presence of ABX, but GD2/GD3 cells containing L444P mutation in compound heterozygosity and with residual GCase activity of 5% showed an increase of GCase activity [14]. However, Kim and coauthors demonstrated that transfected COS-7 cells expressing L444P mutation had an enhanced GCase activity when cultured with ABX [24]. In the same paper, the authors presented four GD3 patients, one being a compound heterozygous for L444P mutation. After 4.5 years of ABX treatment, all reported patients demonstrated clinical benefit from ABX treatment, mainly as better seizure control but without improvement in saccadic eye movement, standing, or walking balance [24]. Results presented in our paper demonstrate the positive enzyme enhancement effect of ABX on GCase activity in L444P/H225Q;D409H fibroblasts and leukocytes. These results support the use of ABX as an add-on treatment to ERT in patients with L444P/H225Q;D409H genotype and possibly other compound heterozygous with L444P allele.

Long-term clinical outcomes of our patients support the efficacy of ABX treatment on neurological features. With ABX treatment, sibling 1 experienced initial improvement and further stable clinical course without neurological deterioration. It has been already reported that ABX might have a positive effect on epilepsy control in GD3 patients [16,25]. In our patient, VPA treatment started around the same time as ABX therapy and therefore it was difficult to conclude if either VPA or ABX alone, or the synergistic effect of both, resulted in good epilepsy control. However, it is important to emphasize that our patient has been seizure-free since ABX treatment was initiated. Initial improvement and subsequent arrest of neurological disease progression are likely attributable to ABX.

Long-term outcome of sibling 2, in whom ABX treatment started in early infancy, before the onset of neurological symptoms, is more remarkable, especially when compared with the clinical course of sibling 1. There has been a recent report on a patient with GD2 detected by newborn screening in whom ABX treatment started at eight months, but at that time patient already had neurological involvement (trunk rigidity and horizontal gaze palsy). Nevertheless, that patient manifested slower neurological deterioration than expected considering genotype, probably due to positive effect of ABX [26]. Sibling 2 has been on ABX treatment since the age of seven weeks, and at the last evaluation, at seven years, the patient had no signs of neurological disease except for low average cognitive functioning and ADHD which were non-progressive and

therefore not necessarily related to GD3. Sibling 2 had a remarkably different clinical course than sibling 1 who manifested the first neurological symptoms at age two years, and by age five years had significant neurological involvement. It is very likely that the good outcome of sibling 2 is attributable to early ABX treatment.

However, we need to be careful when explaining different clinical course in two siblings due to possible genetic and non-genetic modifiers. It has been reported that siblings with GD can have a different phenotype, including the absence of neurological symptoms in siblings of severely affected GD3 patients who harbor the same genotype [8,27]. With regard to possible modifiers or environmental factors that could have contributed to the earlier onset of neurodegeneration in sibling 1, we do not think that the congenital heart defect and open-heart surgeries contributed to the neurological symptoms because these symptoms were GD-specific and the heart-related procedures were uneventful. The co-occurrence of GD and congenital heart disease is very likely coincidental, although this rare combination (GD with tricuspidal atresia) was reported in a French patient more than 50 years ago [28]. Unfortunately, the co-occurrence of a heart anomaly and ascribing the first symptoms and signs of GD to complications of the heart condition postponed the GD diagnosis in sibing 1.

Treatment with ERT and ABX in both siblings resulted in decreased CHT serum activity that was further maintained within the reference range. It is impossible to distinguish the contribution of positive effects of ABX on CHT activity in serum, but lower activities of CHT in the CSF after three months of ABX treatment observed in both siblings could be due to the positive effect of ABX in the CNS. CHT in the CSF represents activated microglia as there are few exogenous monocyte and macrophage cells in the CNS. Olsson and co-authors showed that CHT in the CSF of patients with Alzheimer dementia had a stable activity during the six-month interval, which suggested that pre-analytical variations and imprecisions of the assay had little effect on CHT activity in the CSF. In the same study, CHT activity in the CSF of patients with multiple sclerosis was shown to correlate with the changes of neuroinflammatory status, i.e. disease-modifying treatment significantly reduced CHT [29]. These data encourage us to assume that lower CHT activities in the CSF of both siblings three months after the onset of ABX treatment reflected reduced microglia activation due to the ABX effect. However, the following measurements didn't show a decreasing trend. We don't have an explanation of why CHT activities in the CSF of both siblings increased after an initial decrease. Somewhat reassuring finding was the secondary decline of CHT activity in the CSF of sibling 2 after nine months of ABX treatment. Unfortunately, we don't have additional data for CHT activitiy in the CSF as further analyses were not possible due to parental dissent for the lumbar puncture.

Similar to the CHT activity in the CSF after three months of ABX treatment, the concentration of Lyso-Gb1 in the CSF also decreased considerably in sibling 1. Declining concentrations of Lyso- Gb1 in the serum of both siblings following the initiation of treatment might be a result of the combined effect of ABX and ERT. Several reports demonstrated an additional decrease of Lyso-Gb1 concentrations after ABX treatment was started in patients who were on long-term ERT treatment [24,30]. Hence, Lyso-Gb1 might be a useful biomarker to evaluate the effect of ABX treatment in GD3 patients.

Our patients have been receiving a high dose peroral ABX for seven years without significant side effects; therefore, in this report we bring additional evidence of long-term safety of ABX treatment.

In conclusion, this is, to our knowledge, the first report on long-term safety and efficacy of ABX treatment in a GD3 patient commenced in early infancy, before occurrence of neurological symptoms. Our data support the thesis that early-onset ABX treatment in GD3 patients may postpone or even arrest neurological disease. Further studies are needed to provide more evidence that in some patients with neuronopathic forms of GD neurological symptoms may be preventable if ABX treatment starts early enough. In patients who have already manifested neurological symptoms, ABX treatment may hamper further neurological deterioration. Cultured skin fibroblast assays may be used to evaluate the efficacy of ABX as EET in patients with neuronopathic forms of GD.

Declaration of competing interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2021.03.013.

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Authors' contributions

DPR and MZ wrote the manuscript. IB formulated the idea of the manuscript. KF, MZ and AS performed laboratory diagnostics and fibroblast tests. DPR, TZ, GM and IB were involved in the patient management. AB performed psychological assessments. KBN performed VEP analyses. DO performed neuroimaging. KO provided ABX treatment and follow-up assesment protocols. All authors provided critical feedback and approved the final version of the manuscript.

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