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Comparative case series demonstrating disease modifying outcome with early ambroxol and enzyme replacement therapy in acute neuronopathic Gaucher disease

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Abstract

Gaucher disease (GD) is an autosomal recessive inborn error of metabolism caused by acid β -glucocerebrosidase deficiency, which presents with a wide spectrum of clinical manifestations, ranging from perinatal-lethal to asymptomatic forms. GD type 1 (GD1) is the most frequent visceral form, whereas types 2 (GD2) and 3 (GD3) are associated with neurodegeneration (neuronopathic GD, nGD) and share overlapping manifestations, presenting as a continuum of neurological disease severity. Traditionally, GD2 is the most severe form and has a rapidly progressive course with death by age two to four years. Currently available treatments for GD include enzyme replacement therapy and substrate reduction therapy to ameliorate visceral, hematologic, and skeletal abnormalities; however, they are not efficacious for neurological manifestations. More recently, ambroxol has been



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suggested as an augmentative pharmacological chaperone for nGD. This comparative case series of three patients aims to demonstrate the impact of early neonatal initiation of ambroxol on neurological outcomes in our index case (Patient A), compared to two other patients (Patients B and C) who began ambroxol treatment at 10 months and 4 years and 1 month of age, respectively, after a later diagnosis of nGD with already established neurological symptoms. Patient A has normal age-related developmental milestones at four years, while Patients B and C have persistence of developmental delay albeit some forward progression in the former. Our report highlights that early neonatal diagnosis and ambroxol therapy could potentially be a game changer in mitigating disease progression in nGD by modifying the natural history of GD2. Expanding newborn screening will facilitate early diagnosis and significantly impact clinical outcomes and quality of life in GD patients with early initiation of treatment.

Keywords: Acute neuronopathic Gaucher disease, ambroxol, enzyme replacement therapy, disease-modifying outcome

INTRODUCTION

Gaucher disease (GD) is a rare autosomal recessive lysosomal storage disorder caused by deficient activity of β -glucocerebrosidase (GCase; EC 3.2.1.45), which hydrolyses glucosylceramide (GluCer) into glucose and ceramide. Biallelic pathogenic variants in *GBA1* lead to misfolding of GCase in the endoplasmic reticulum, defective trafficking and targeting to the lysosomes, and progressive accumulation of GlcCer with its deacylated derivative, glucosylsphingosine (GlcSph), in lysosomal macrophages known as Gaucher cells in the reticuloendothelial system, bones, and central nervous system^[1,2]. GD presents as a clinical spectrum ranging from a severe, perinatal-lethal phenotype - often seen in collodion babies - to acute or chronic neuronopathic forms, as well as non-neuronopathic GD, which may involve skeletal and visceral manifestations, Parkinson's disease, or remain asymptomatic into adulthood^[2]. Although the phenotype is variable, three clinical types have been historically identified: type 1 (non-neuronopathic form, MIM# 230800), characterized by the presence of primary central nervous system involvement; type 2 (acute neuronopathic form, MIM# 230900), marked by early and severe neurological symptoms; and type 3 (chronic neuronopathic form, MIM# 231000), which features later-onset and more slowly progressive neurological involvement. GD type 1 (GD1) is the most common and its manifestations are limited to the skeletal and reticuloendothelial systems with the absence of neurological symptoms^[1]. Although GD1 has typically been described as a non-neuronopathic form, there is increasing evidence of neurological involvement with parkinsonian syndrome and Lewy body dementia in affected patients^[3]. GD types 2 (GD2) and 3 (GD3) are characterized by the presence of primary neurologic disease and may be distinguished by the age of onset and rate of disease progression. Individuals with GD2 have early-onset disease before age two, limited psychomotor development, and death in infancy (median nine months)^[4], or early childhood, by age two to four years^[2]. GD type 3 may present before age two years, but often has a more slowly progressive course, with survival into the third or fourth decade^[5]. Assigning a definitive diagnosis for the neuronopathic GD (nGD) subtypes can be challenging in very young patients. Very rarely, deficiency of GCase activity occurs as a result of biallelic pathogenic variants in the *PSAP* gene, leading to a deficiency in the GCase activator saposin C (MIM# 610539)^[6] and clinical features characteristic of severe nGD or GD1^[2]. Saposin C is one of four homologous proteins, referred to as saposin A-D, which arise from proteolytic cleavage of the saposin precursor protein, prosaposin^[7]. Affected individuals with saposin C deficiency demonstrate glucosylceramide accumulation and visceromegaly, but have normal *in vitro* GluCase activity^[2].

The incidence of GD1 is approximately 1 in 60,000 globally, rising to 1 in 800 to 1 in 950 in the Ashkenazi Jewish community^[4]. A Japanese study reported a prevalence of 1:530,000, with approximately 38% of patients having GD1, while around 54% have GD2 or GD3^[5]. The prevalence of GD in the Australian

population during an 11-year period from January 2009 through to December 2020 was 1 in 74,000 live births^[8]. Traditionally, the diagnosis of GD can be confirmed by demonstrating reduced GCase activity in peripheral blood leukocytes, followed by the identification of biallelic pathogenic variants in *GBA1*. The presence of a pseudo-deficiency allele has been described as a possible confounder in the interpretation of enzymatic assay results in newborn screening programs, which have reported false positives based on decreased GCase activity^[9,10]. More recently, GlcSph has been shown to be a key diagnostic biomarker of GD with 100% specificity and sensitivity to distinguish GD patients from unaffected individuals and a variety of related inherited metabolic disorders^[11]. Additionally, its utility as a pharmacodynamic marker in evaluating therapeutic response and its prognostic attribute by differentiating neuronopathic (nGD) from their non-neuronopathic counterparts are valuable. The applicability of GlcSph could be expanded to second-tier testing following multiplexed enzymology in newborn screening^[11,12].

The introduction of enzyme replacement therapy (ERT) in 1991 has revolutionized the management of GD1^[13]. Currently, three recombinant glucocerebrosidase enzyme preparations available include imiglucerase (Cerezyme®), velaglucerase alfa (VPRIV®), and taliglucerase alfa (Elelyso®)^[2]. ERT has been shown to improve hematopoietic reconstitution, resulting in a reduction in liver and spleen volumes and an improvement or stabilization of skeletal abnormalities^[2]. However, ERT alone does not alter the trajectory of neurologic disease in GD, although it can result in significantly enhanced quality of life in GD3 due to improvement in systemic manifestations^[2]. The International Collaborative Gaucher Group consensus recommendation for GD1 includes early initiation of treatment for all symptomatic children and adolescents, while enlisting a “watchful waiting” approach restricted to asymptomatic adults with genotypes associated with mild disease severity^[14].

Substrate reduction therapy (SRT) is a newer therapeutic option aimed at decreasing the amount of substrate precursor (GluCer) synthesized to a level at which the patient’s own residual GCase activity can effectively clear the glycosphingolipids and reduce storage burden^[15]. Miglustat (Zavesca®, Actelion) is the first oral agent for the treatment of individuals with mild to moderate GD1 for whom ERT is not a therapeutic option due to intolerance or poor venous access. Miglustat has not been found to have any effect on neurological symptoms in GD3, despite its ability to cross the blood-brain barrier^[1]. Eliglustat, an alternative glucosylceramide synthetase inhibitor, has been reported to improve hematologic and visceral parameters for GD1 adults with extensive, intermediate, or poor cytochrome P450 2D6 (CYP2D6) metabolizer status (> 90% of patients). Eliglustat is metabolized by CYP2D6 and, to a lesser extent, CYP3A; hence, its dosage is based on the individual’s CYP2D6 metabolizer status^[16,17].

No existing treatment is effective for nGD due to the difficulty in delivering therapies across the blood-brain barrier^[18]. Early diagnosis and supportive treatment are imperative but often delayed due to the rarity of the disease coupled with the lack of awareness among clinicians. Since the last decade, ambroxol has gained the spotlight for its potential as a chaperone treatment of GD, predominantly for neurological and skeletal manifestations^[19,20], while also being effective for visceral and hematological complications. Traditionally, ambroxol has been used as an oral mucolytic drug available over the counter for many years as a cough medicine. It is also a potentiator of surfactant and has been utilized for hyaline membrane disease in neonates. Chaperone therapy (CT) is proposed to correct misfolded GCase enzyme to improve its function and facilitate transport across the blood-brain barrier, which is not possible with ERT or SRT. Ambroxol was identified as a potential CT through screening of an established drug database^[18]. Several case studies with limited patient cohorts have reported its potential to improve and stabilize neurological and skeletal manifestations, with and without ERT^[19-31]. While these case reports highlight promise for GD patients with progressive neurological manifestations, ambroxol has been commenced in all but one of these case studies

on patients after neurological and skeletal symptoms have been established. A single report described the commencement of ambroxol on an asymptomatic patient^[24]. In this comparative case series, we describe, to the best of our knowledge, the youngest GD2 patient treated with ERT and ambroxol from the neonatal period, and compare his outcome to later initiation in two other nGD patients from our center, to demonstrate its clinical efficacy in delaying the onset of debilitating neurological symptoms and improving quality of life when used as adjunct therapy with ERT for treatment of visceral manifestations.

Clinical summary

Patient A

A male neonate born at term in a non-tertiary hospital to non-consanguineous Caucasian parents via vaginal delivery had Apgar scores of 9, 8, and 9 at 1, 5, and 10 min, respectively. The pregnancy was complicated by gestational thrombocytopenia and iron deficiency anemia requiring multiple transfusions. Ten hours post birth, he developed respiratory distress secondary to hepatosplenomegaly (liver 6-7 cm and spleen 8-9 cm below the costal margin), associated with significant diaphragmatic splinting, which was managed with continuous positive airway pressure (CPAP). Blood film showed a leucoerythroblastic picture and laboratory tests demonstrated thrombocytopenia, anemia, and liver dysfunction requiring transfer to a tertiary-level neonatal intensive care unit where multiple transfusions of platelet and packed red blood cells were administered. His platelet had a nadir of $25 \times 10^9/L$ with a hemoglobin nadir of 88 g/L. Initial liver function test showed elevated transaminases of AST 698 u/L (reference range 0-213) and ALT 161 u/L (reference range 0-58). The hepatosplenomegaly was confirmed on abdominal MRI with a liver span measuring 8.3 cm and a spleen of 9.8 cm. He was not able to be weaned off CPAP due to recurrent central apnoea noted on trolley and sleep studies. EEG performed for suspicious involuntary movements failed to reveal any epileptiform discharges; however, slightly excessive asynchrony was observed in sleep, more than would be expected for gestational age. Expedited confirmatory molecular testing performed on day six of life with trio whole-genome sequencing identified compound heterozygous pathogenic variants (c.887G>A, p.Arg296Gln and c.1342G>C, p.Asp448His) in *GBA1* [Table 1]. GCase activity in leucocytes was 1.4 nmol/h/mg protein (reference range 6.5-15), and the baseline plasma GluSph level was 1,450 nmol/L (reference range ≤ 10). Markedly elevated plasma GluSph and the early, severe clinical phenotype were consistent with acute neuronopathic GD2. However, his MRI brain was normal, and there were no clinical signs of bulbar dysfunction, horizontal ophthalmoplegia, abnormal posturing, myoclonus, or seizures commonly present in GD2^[1,32].

He was commenced on oral ambroxol at two weeks of age at 3 mg/kg/day, which was incrementally titrated to approximately 25 mg/kg/day over a 3-month period. Enzyme replacement therapy was commenced with fortnightly velaglucerase alfa (VPRIV®) at 60 IU/kg/dose. The neonatal period was complicated by methicillin-sensitive *Staphylococcus aureus* central line sepsis on day 24, for which he was treated with intravenous antibiotics and recovered. He was discharged to his local hospital at five weeks of age on CPAP and nasogastric (NG) feeds due to poor sucking reflex. He showed gradual improvement over the next few weeks and was weaned off NG feeds by six weeks, and CPAP by four months of age after commencing ambroxol.

Since the commencement of combined therapy, serial plasma GluSph levels monitored demonstrated significant improvements, with an 88% reduction to 170 nmol/L after two months of therapy and a total reduction of 97% to 40 nmol/L after one year on combined therapy [Table 1]. We used plasma GluSph level as a marker of disease in lieu of cerebrospinal GluSph as this assay is not available as a clinical test in Australia. Notably, there was complete resolution of respiratory compromise, hepatomegaly, and cytopenias. Hematological and hepatic markers have normalized. Magnetic resonance imaging (MRI) of the brain at 10 weeks of age was normal. A formal developmental assessment at five months of age using Bayley

Table 1. Comparative data of our three patients

	A	B	C
Gender	M	M	F
Current age (months)	48	35	101
Age at diagnosis	Day 6	10 months	16 months
Clinical presentation	Hepatosplenomegaly, thrombocytopenia, anemia, recurrent central apnoea	Global developmental delay, central hypotonia, regression, mild splenomegaly, faltering growth, apnoea/upper airway obstruction	Hepatosplenomegaly, oculomotor apraxia, thrombocytopenia
Clinical features			
Neurological	Nil post ABX	Gross and fine motor delay, expressive language delay, irritability, oculomotor apraxia	Seizures, ataxia, dystonia, severe global developmental delay
EEG	Normal	N/A	Abnormal- frequent spike and wave discharges from bilateral central and vertex leads
Ophthalmological	Nil	Oculomotor apraxia	Oculomotor apraxia
MRI Brain	Normal	Non-specific diffusion restriction of fimbria-fornix complex bilaterally, optic chiasm and parts of adjacent bilateral optic nerves and tracts	Slightly prominent left lateral ventricle and CSF spaces over the left cerebral hemisphere, no diffusion restriction
Liver span (US) (cm)			
• Pre-treatment	7.4	8	11.4
• Post-treatment*	10.2	8.5	8.5
Spleen span (US) (cm)			
• Pre-treatment	9.1	11	12.5
• Post-treatment*	10.2	14.2	8
<i>Skeletal</i>	Nil	N/A	Nil
<i>Pulmonary</i>	Nil	N/A	Nil
Hematology			
Hemoglobin (g/L)			
• Pre-treatment	72	98	105
• Post-treatment*	120	107	130
Platelets ($\times 10^9$ /L)			
• Pre-treatment	7	155	100
• Post-treatment*	248	214	239
White cell count ($\times 10^9$ /L)			
• Pre-treatment	3.9	8.7	4.7
• Post-treatment*	8.7	7	6.9
Biochemical and Genomic			
GCase activity (6.5-15 nmol/h/mg) [#]	1.4	1.4	0.3
Glucosylsphingosine (< 10 nmol/L)			
Pre-treatment	1,450	840	1,200
Post-treatment*			
• Ambroxol only	-	280	N/A
• ERT only	-	N/A	170
• Combined ERT and ambroxol	40	80	130
Genotype (GBA1 variant)		NM_000157.3	NM_000157.3

• Allele 1	c.887G>A; p.Arg296Gln (P)	c.882T>G; p.His294Gln (P) c.1342G>C; p.Asp448His (P)	c.689T>G; p.Val230Gly (V191G) (LP)
• Allele 2	c.1342G>C; p.Asp448His (P)	c.721G>A; p.Gly241Arg (P)	c.1448T>C; p.Leu483Pro(L444P) (P)
<i>Treatment</i>			
Age at initiation of ERT	2 weeks	29.5 months	19 months
Age at initiation of Ambroxol	2 weeks	10 months	49 months
<i>Duration of Therapy (Months)</i>			
• Ambroxol (ABX)	47+	25	52
• ERT	47+	5	82
• Combined therapy	47+	5	52
Ambroxol (maximum dose)	30 mg/kg/day	28 mg/kg/day	25 mg/kg/day
ERT dose	60 units/kg fortnightly	60 units/kg fortnightly	60 units /kg fortnightly
<i>Treatment Outcome</i>			
Developmental assessment	Bayley Scales of Infant and Toddler Development Australian and New Zealand 4th ed- very high scores in fine motor skills, high average in cognition and gross motor. Clinically, developmental milestones are age-appropriate at 4 years	Griffith Scales for Child Development performed at 2 years 4 months consistent with global developmental delay	At 55 months - adaptive ability - < 1st percentile (low range); intellectual disability, severe range
Neurological outcome	Weaned off NG feeds 3 weeks and cessation of central apnoea 3+months post ABX. Normal development to date	Decreased apnoeic episodes and subsequent complete cessation within 3 and 6 months, respectively, of ABX therapy. Making slow developmental progress, walking independently, good receptive language, ongoing expressive language delay, convergent strabismus	Oculomotor apraxia, seizures and epileptiform on EEG, severe global developmental disability, ataxia and dystonic/hyperkinetic movements. Post ABX, the rate of neurological decline decreased; seizures stabilized within the first year, with cessation by 24 months of treatment. Recurrence of seizures 33 months post ABX

*Most recent systemic and hematological parameters; #Measurement of acid β -glucocerebrosidase activity in tissue leukocytes^[9]; ^Quantification of plasma glucosylsphingosine^[11].

Scales of Infant and Toddler Development Australian and New Zealand Fourth Edition (Bayley-4) demonstrated scores in the high average range in receptive communication, fine and gross motor domains and average in cognition and expressive communication. A repeat Bayley-4 at 24 months reported above-average scores in his fine motor skills, and high-average in his cognition and gross motor skills. His developmental milestones are age-appropriate when last reviewed at four years of age. He has not displayed any bulbar dysfunction or oculomotor apraxia. He has tolerated ambroxol and velaglucerase without adverse effects.

Patient B

Patient B first presented at the age of eight months with a diarrhoeal illness in the setting of poor weight gain, recurrent respiratory infections, and irritability over the preceding four weeks. He had intermittent mild upper airway obstruction and was diagnosed with laryngomalacia. History of gross motor delay, central hypotonia, and possible motor regression were noted. The liver edge was palpable 3 cm below the costal margin. Abdominal sonogram demonstrated mild splenomegaly with normal liver size. Neurological examination revealed head lag and intermittent esotropia.

He was readmitted at nine months with increasing upper airway obstruction and accompanying cyanosis. Flexible nasendoscopy demonstrated paradoxical vocal cord movements. MRI of the brain was normal, and

the echocardiogram had not identified structural cardiac abnormalities. Chest radiographs showed persistent pulmonary infiltrate.

White cell enzyme panel for lysosomal disorders returned a low GCase activity, 1.4 nmol/h/mg protein (reference range 6.5-15), and elevated plasma GluSph 840 nmol/L (reference range < 10). Sequencing of *GBA1* identified three disease-causing variants [Table 1] inherited from both parents, which confirmed the diagnosis of GD. These results, in combination with his clinical phenotype, are consistent with neuronopathic GD.

He was commenced on ambroxol 3 mg/kg/day at 10 months of age, which was gradually increased to a target dose of 25 mg/kg/day over the next 10 months. Plasma GluSph levels subsequently decreased to 280 nmol/L (reference range < 10). Oral gabapentin was used for the management of significant irritability. These combined therapeutic approaches ameliorated his neuro-irritability. His apnoeic episodes decreased within three months and completely ceased six months post ambroxol therapy. He also demonstrated developmental gains, although he continued to perform below the expected range for age. He sat independently after one year of age and walked with a broad-based gait at around 2 years. He communicated with gestures at 18-20 months and vocalized at two years. Griffith Scales of Child Development 3rd Edition administered at two years and four months demonstrated below-average developmental milestones across all domains. ERT was commenced at 29 months of age for progressive somatic symptoms. Plasma GluSph levels decreased further to 80 nmol/L (reference range < 10). Based on his initial presentation with global developmental delay, central hypotonia, and paradoxical vocal cord movements at eight to nine months of age, his clinical phenotype is consistent with GD2.

Patient C

A female infant presented at 16 months of age with a preceding history of oculomotor apraxia from eight months of age, followed by hepatosplenomegaly and thrombocytopenia at the time of diagnosis. GCase activity was markedly reduced at 0.3 nmol/h/mg and GlcSph levels were significantly elevated at 1,200 nmol/L. *GBA1* variant testing with subsequent parental segregation identified compound heterozygous variants [Table 1]. ERT was initiated at 19 months of age. By 43 months, her developmental progress had plateaued, and she became increasingly ataxic with the emergence of dystonia, hypertonic movements, and later, seizures. Ambroxol was commenced at 49 months of age, with continuation of ERT. Plasma GluSph levels decreased to 130 nmol/L (reference range < 10). Now, at eight years of age, her neurological symptoms persist; however, the rate of neurological decline has decreased, and seizures stabilized within the first year of ambroxol therapy, ceasing entirely after 24 months. She has an intellectual disability with speech delay, persistent oculomotor apraxia, and new onset of seizures with EEG demonstrating epileptiform discharges at 33 months, which necessitates antiepileptic therapy. She has persistent dystonia, worse in the lower limbs. Her visceral surveillance investigations show a reduction in the liver and spleen spans and an improvement in hemoglobin and platelet counts over the course of treatment. Clinically, her phenotype is consistent with GD3.

DISCUSSION

Ambroxol has gained attention in the last decade for its potential to improve neurological and skeletal manifestations (not possible with ERT alone) due to its ability to cross the blood-brain barrier as demonstrated in the mouse model^[33]. In GD, the mutant enzyme misfolds, accelerating endoplasmic reticulum (ER)-associated degradation, despite the maintenance of functional potential^[20]. As a pharmacological chaperone, ambroxol selectively binds to misfolded GCase in the neutral pH environment of the ER, promoting proper protein folding. It then dissociates from the enzyme in the acidic pH

environment of the lysosome, thereby enabling functional recovery^[21]. GCase activity has been shown to be increased in the cerebellum, heart, and spleen of GD mice treated with ambroxol^[34] and in the midbrain, cortex, cerebellum, and striatum in non-human primate models^[35,36]. Furthermore, fibroblast studies have provided insight into genetic variants of *GBA1* that have shown promising response to ambroxol, including F213I, N370S, R131C, N188S/G193W, R120W/L444P, R120W/N370S, L444P/L444P, F213I/L444P, P415R/L444P, F213I/RecNcil, D409S/IVS10G>A, although conflicting data for several genotypes have demonstrated varying outcomes in fibroblast studies^[21,34,37-42]. Variable outcomes of *in vitro* studies suggest that alternative intracellular processes intrinsic to Gaucher cells potentiate the effect of ambroxol and highlight the need for further studies to elucidate the precise mechanism of action. Importantly, mutations without confirmed *in vitro* response have also shown *in vivo* increases in GCase. More than 65% of 40 nGD patients had responded favorably to high-dose ambroxol therapy, irrespective of genotypes^[29].

The first pilot study, conducted to assess the tolerability and efficacy of ambroxol on 12 symptomatic GD1 patients (mean age: 41.1 years; range 24-63), involved treatment with 150 mg of ambroxol daily for six months. The results showed increased platelet counts and reduced spleen volumes, while hemoglobin levels and liver volumes remained stable^[43]. One patient with pulmonary hypertension showed no clinical worsening during the six-month treatment period but experienced deterioration after discontinuing ambroxol. Among the three patients who chose to continue treatment for an additional six months beyond the initial study period, similar further clinical improvements were observed. A subsequent proof-of-concept study evaluated the efficacy, safety, and tolerability of high-dose ambroxol (25 mg/kg/day or maximum dose 1,300 mg/day) in combination with ERT in five patients with neuronopathic GD, aged 5 to 28 years. The study demonstrated marked improvements in myoclonus, seizures, and pupillary light reflex dysfunction in all patients, with notable recovery of gross motor function in two patients, enabling them to walk again. Ambroxol was well tolerated by all patients, and no adverse effects were reported^[20]. Several case reports since then have described variable benefits of ambroxol for progressive neurological symptoms in Gaucher disease [Table 2 and Table 3]. However, a similar beneficial response was not observed in a pair of twin siblings with an intermediate phenotype between GD2 and GD3, characterized by complex neurological symptoms and parkinsonian features. Ambroxol, administered alongside ERT, was discontinued within one month of initiation due to increased tremors and axial instability noted by the parents. Both siblings subsequently responded to levodopa, showing reduced tremors and improvements in motor skills and cognitive function^[44].

Ambroxol supplementation combined with ERT in two Canadian patients with GD3 led to initial clinical improvements in ambulation in both patients; however, these improvements were later lost in the first patient due to fractures. No notable changes were observed in seizures in either patient; nonetheless, seizure duration was substantially reduced in the second patient (N188S/R463H in *GBA*), eliminating the need for hospital admissions due to status epilepticus over a three-year period and thereby enhancing her quality of life. The variability in response to ambroxol between the two patients was proposed to be associated with their different genotypes^[23]. This hypothesis was supported by a subsequent study highlighting the benefits of *in vitro* testing of fibroblasts to determine individualized response prior to *in vivo* trial with high doses of ambroxol in two patients with GD3 (25 mg/kg/day and 1,300 mg/day, respectively) on ERT^[25]. In a patient with compound heterozygous variants, N188S and a null allele (IVS2 + 1G > A), ambroxol therapy initiated at 14.5 years resulted in a 67% increase in enzymatic activity in cultured fibroblasts from a baseline of 26.5 nmol/mg/h (normal: 86.12 ± 25.00). Correspondingly, *in vivo* improvements were observed in epilepsy and GD biomarkers. In the second patient, homozygous for the L444P variant, with the residual enzymatic activity of 12.8 nmol/mg/h in cultured fibroblasts, ambroxol started at age 60 was clinically ineffective. The authors suggest that additional factors beyond *GBA1* mutations - such as variations in the complex ER network involved in proteostasis - may influence therapeutic response to ambroxol^[25].

Table 2. Characteristics of previously reported nGD patients and their response to Ambroxol

	Age at presentation with nGD	Age at diagnosis	Sex	GD type	Glucocerebrosidase activity	GluSph level (nmol/L) - plasma/DBS/CSF	GBA1 Variant	Neurological symptoms	Age ABX commenced	Combined ERT & ABX	Outcome post ABX
Patient 1 ^[25]	NA	6 years	F	III	Fibroblasts - 26.5 nmol/mg/h (RR: 86.12 ± 25.00)	31.4 ng/mL (P)	IVS2+1G>A/N227S	Pathological EEG from 14 years, frequent seizures, abnormal saccadic eye movements	14.5 years	Yes (ERT commenced at 6 years)	Reduced seizure frequency, antiepileptic dosing. Unchanged saccadic eye movements
Patient 2 ^[25]	NA	14 years	M	III	Fibroblasts -12.8 nmol/mg/h (RR: 86.12 ± 25.00)	NA	L483P/L483P	Drug-resistant epilepsy at 16 years, mild intellectual disability, mild camptocormia, gait abnormality	60 years, gradually titrated to a maximum of 1,300 mg/daily	Yes (ERT commenced at 37 years)	No improvements. ABX ceased after 4 months of maximal dose
Patient 3 ^[22]	Collodion baby	Neonate	F	II	Lymphocytes -newborn- 0.1 μmol/l/h - RR > 3.2 μmol/l/h. DBS, 3 months 52.65 pmol/spot 20 h (RR -200-2,000 pmol/spot 20 h)	322 ng/mL (P); (908 pg/ml (CSF)	R398L/R398L	Increased tone and hyperexcitability at 3 months	4 months, commenced at 25 mg/kg/day	Yes (ERT commenced at 15 months)	Improved overall quality of life. Slight cognitive delay at 34 months. Reduction in CSF GluSph. Increase GCcase lymphocyte and <i>in vitro</i> fibroblast study
Patient 4 ^[26]	10.4 years	NA	F	nGD	Collective results provided for all 4 patients as a range	Collective results provided for all 4 patients as a range	N227S/R296Q	Myoclonus, generalized tonic-clonic seizures, intellectual disability	15.6 years	Yes (prior to ABX commencement)	Improved swallow, speech, pyramidal, extrapyramidal symptoms. Decreased myoclonic, GTC seizure frequency. Standing and walking balance recovered. Deteriorated horizontal and vertical saccadic movements. Significantly increased GCcase activity, reduction in plasma GluSph
Patient 5 ^[26]	10.1 years	NA	F	nGD	Collective results provided for all 4 patients as a range	Collective results provided for all 4 patients as a range	N227S/R296Q	Myoclonus, generalized tonic-clonic seizures, intellectual disability	12.8 years	Yes (prior to ABX commencement)	Improved swallow, speech. Decreased myoclonic, GTC seizures. Deteriorated horizontal and vertical saccadic

											movements, standing and walking balance. Significantly increased GCcase activity, reduction in plasma GluSph
Patient 6 ^[26]	10.5 years	NA	F	nGD	Collective results provided for all 4 patients as a range	Collective results provided for all 4 patients as a range	N227S/V211fs	Myoclonus, generalized tonic-clonic seizures, intellectual disability	10.1 years	Yes (prior to ABX commencement)	Improved epilepsy, cerebellar tremor. Unchanged standing, walking balance, horizontal and vertical saccadic movement. Significantly increased GCcase activity, reduction in plasma GluSph
Patient 7 ^[26]	13.1 years	NA	F	nGD	Collective results provided for all 4 patients as a range	Collective results provided for all 4 patients as a range	F252I/L483P	Myoclonus, intellectual disability	12.7 years	Yes (prior to ABX commencement)	Improved epilepsy, cerebellar tremor. Stationary horizontal and vertical saccadic movements. Unchanged standing and walking balance. Significantly increased GCcase activity, reduction in plasma GluSph
Patient 8 ^[23]	10 years	1 year, diagnosed with GD1	M	III	1.62 μ mol/h/mg protein (RR: 15.2 $\pm$ 6.3 nmol/h/mg protein)	35.4ng/mL, RR $\leq$ 4.8 (DBS) at ~ 12 years	G416S/G282E	Refractory epilepsy at 10 years, unsteady gait at 11 years, wheelchair dependent at 12 years	12 years, commenced at 25 mg/kg/day	Yes (ERT commenced at 1 year)	Improved ataxia, ambulation without wheelchair. Unchanged seizures, EEG. Reduction in DBS and CSF GluSph
Patient 9 ^[23]	10 years	3 years, diagnosed with GD1	F	III	0.6 μ mol/h/mg protein, RR: 15.2 $\pm$ 6.3 nmol/h/mg protein	31ng/mL, RR $\leq$ 4.8 (DBS) at 18 years	N227S/R502H	Refractory epilepsy, progressive ataxia, tremor, wheelchair-bound at 18 years with frequent episodes of status epilepticus	18 years, commenced at 25 mg/kg/day	Yes (ERT commenced at 3 years)	Improved ataxia, ambulation without assistance. Reduction of seizure duration but unchanged type and frequency. Reduction in DBS GluSph
Patient 10 ^[30]	NA	2 years	F	III	NA	NA	NA	Epilepsy at 20 years, became drug-resistant	35 years, commenced at 4 mg/kg/day	Yes (ERT commenced at 19 years)	Significant reduction of seizures
Patient	Neonate	Neonate	F	II/severe	0.47 μ M/h	117.88 ng/mL (N < 3)	L483P/RecNcil	Truncal rigidity and mild	8 months,	Yes (ERT	Strabismus, gross

11 ^[24]	(day 6)		III	N > 1.8 μ M/h	(P)			horizontal gaze palsy at 6 months. Strabismus at 11 months. Delayed gross motor development and ataxia at 18 months	commenced at 30 mg/kg/day	commenced at 5 weeks) for low platelet count, borderline splenomegaly implying a severe phenotype	motor delay at 18 months. No developmental regression or seizures
Patient 12 ^[27]	2 months	5 months	F	III	Low- not specified	NA	L483P/L483P	Horizontal gaze oculomotor	5 months, commenced at 15 mg/kg/day	Yes, simultaneously	Age-appropriate developmental milestones. Blood counts normalized after starting ERT
Patient 13 ^[20]	12 years	20 years	F	III	Lymphocytes-3.2 nmol/h/mg protein (13.7% control)	CSF- 18.2 pg/mL (RR < 10.0)	N227S/G232W	myoclonic-generalized status epilepticus, daily seizures, gaze palsy, bed-bound, gavage feeding, tracheostomy, non-verbal	28 years, commenced at 3 mg/kg/day to reach a target dose of 25 mg/kg/day	Previous ERT for 8 years	Improvement of facial myoclonus, reduced seizure frequency, improved pupillary light reflex (PLR), horizontal saccadic latency
Patient 14 ^[20]	7 years	19 years	F	III	Lymphocytes-5.8 nmol/h/mg protein (24.7% control)	CSF- 26.6 pg/mL (RR < 10.0)	N227S/?	Unable to sit without support, use wheelchair since 17 years, myoclonus of limbs, myoclonic seizures, generalized tonic-clonic seizures, horizontal saccadic gaze initiation failure	20 years initiated at 3 mg/kg/day to reach a target dose of 25 mg/kg/day	Previous ERT for 4 months	Improvement of myoclonus, mobility, fine motor skills, PLR, horizontal saccadic latency. Decreased seizure frequency
Patient 15 ^[20]	8 years	14 years	F	III	Lymphocytes-7.1 nmol/h/mg protein (30.1% control)	CSF- 49.1 pg/mL (RR < 10.0)	N227S/?	Barely standing with support for a short period, use of wheelchair since 14 years, myoclonus of trunk and limbs, genealized tonic-clonic seizures, horizontal saccadic gaze initiation failure	15 years initiated at 3 mg/kg/day to reach a target dose of 25 mg/kg/day or a maximum dose of 1300 mg/day	Previous ERT for 4 months	Improvement of myoclonus, mobility and fine motor skills, PLR, horizontal saccadic latency. Reduced seizure duration
Patient 16 ^[20]	3 months	11 months	F	II	Lymphocytes-4.3 nmol/h/mg protein (18.1% control)	CSF- 635 pg/mL (RR < 10.0)	F252I/RecNcil	Horizontal saccadic gaze initiation failure, head thrusting at 3 months. Bedridden, tube feeding at 13 months, tracheostomy and ventilation at 20 months, non-verbal, generalized myoclonus, tonic and generalized	3 years initiated at 3 mg/kg/day to reach a target dose of 25 mg/kg/day	Previous ERT for 2 years	Improvement of myoclonus, PLR, horizontal saccadic latency. Reduced frequency of generalized convulsive status epilepticus

Patient 17 ^[20]	6 months	3 years	F	III	Lymphocytes post bone marrow transplantation - 23.6 nmol/h/mg protein (100.6% control)	CSF- 146 pg/mL (RR < 10.0)	D448H/IVS10-1	tonic-clonic seizures, gaze palsy in all directions Apnoeic spells at 6 months. Bedridden at 18 years, tube feeding and tracheostomy at 25 years, generalized dystonia, myoclonus of limbs and face, generalized tonic-clonic seizures, horizontal saccadic gaze initiation failure, vertical gaze palsy	25 years initiated at 3 mg/kg/day to reach a target dose of 25 mg/kg/day	BMT at 4 years	Improvement of myoclonus, PLR, horizontal saccadic latency, fine motor skills. Unchanged dystonia. Reduced seizure frequency
Patient 18 ^[31]	NA	2 years	M	II	NA	NA	L29fs/V433L	NA	NA	Yes	ABX discontinued due to increased mucous production, cough
Patient 19 ^[31]	NA	2 years	F	II	NA	NA	L483P/IVS2+1	NA	NA	Yes	Improved head movement and contact with parents
Patient 20 ^[31]	NA	1.5 years	F	II	NA	NA	L483P/N227R	NA	NA	No	Patient died
Patient 21 ^[31]	NA	5 years	F	III	NA	NA	L483P/L483P	NA	NA	Yes	Eye movement anomalies persist
Patient 22 ^[31]	NA	20 years	F	III	NA	NA	E272D/L483P	NA	NA	Yes	Near resolution of tremor, improved mobility, fine motor skills, clearer speech, reduced seizure frequency
Patient 23 ^[31]	NA	6 years	M	III	NA	NA	L483P/L483P	NA	NA	Yes	No neurological deterioration
Patient 24 ^[31]	NA	5 years	M	III	NA	NA	L483P/L483P	NA	NA	Yes	No neurological deterioration
Patient 25 ^[31]	NA	4 years	M	III	NA	NA	L483P/L483P	NA	NA	Yes	ABX discontinued due to adverse effects
Patient 26 ^[31]	NA	5 years	F	III	NA	NA	L483P/L483P	Slow horizontal saccadic movements	NA	Yes	No neurological deterioration
Patient 27 ^[31]	NA	12 years	F	III	NA	NA	L483P/L483P	Eye movement anomalies	NA	Yes	No neurological deterioration
Patient	NA	7 years	F	III	NA	NA	L483P/F252I	Gaze palsy	NA	Yes	No neurological

28 ^[31]												deterioration
Patient 29 ^[31]	NA	39 years	F	III	NA	NA	L483P/L483P	NA	NA	Yes	Splenectomized, decreased pain	
Patient 30 ^[31]	NA	19 years	M	IIIc	NA	NA	D448H/D448H	NA	NA	Yes	Discontinued due to reimbursement issues	
Patient 31 ^[31]	NA	17 years	F	IIIc	NA	NA	D448H/D448H	NA	NA	Yes	Discontinued due to reimbursement issues	
Patient 32 ^[31]	NA	5 years	F	IIIc	NA	NA	D448H/D448H	NA	NA	Yes	NA	
Patient 33 ^[31]	NA	12 years	F	III	NA	NA	H294Q/L483P	Supranuclear gaze palsy, impaired fine motor skills, no seizures or spasticity	NA	Yes	Stable neurological symptoms	
Patient 34 ^[31]	NA	12 years	M	III	NA	NA	L483P/L483P	NA	NA	Yes	Increased physical activity, reduced fatigue	
Patient 35 ^[31]	NA	3 years	F	IIIc	NA	NA	D448H/D448H	NA	NA	Yes	NA	
Patient 36 ^[31]	NA	7 years	F	III	NA	NA	L483P/L483P	NA	NA	Yes	No seizures for at least a year	
Patient 37 ^[31]	NA	7 years	M	III	NA	NA	N227S/R159W	NA	0.5	Yes	Improvement in frequency and intensity of seizures. Decreased GluSph	
Patient 38 ^[28]	3 months	4 years 9 months	F	III	0.9 nmol/mg protein/h, RR 5.4-11.6	NA	L483P/H294Q;D448H	Convergent strabismus at 2 years, delayed growth and seizures at 4 years 9 months	5 years	Yes, ERT commenced at ~ 4 years 9 months	Improvement, stabilization of neurological symptoms. Seizure free. Reduced oculomotor apraxia, dysarthria	
Patient 39 ^[28]	6 weeks	2 weeks-	M	III	0.67 nmol/mg protein/h	NA	L483P/H294Q;D448H	No neurological symptoms	7 weeks	Yes, ERT commenced at 3 months	Low average cognitive functioning and ADHD (not necessarily related to GD3) at 7 years	
Patient 40 ^[29]	11 months	15 months	F	II	1.05 pmol/h/disk (RR: 4.1-9.7)	NA	L483P/R502H	Recurrent dysphagia, hypotonia, growth arrest, psychomotor retardation, bilateral ptosis and esotropia, laryngospasm with	15 months	Yes, simultaneously at 15 months	Improvement in motor activity within 1 week of ABX therapy. At 3 years 9 months, walked independently;	

										tracheostomy	cognitive function reached 12-16 months of developmental age. Improvement in quality of life
Patient 41 ^[44]	12 months	18 months	M	II-III	28% (RR < 30% compatible with GD)	NA	D448H/L422Pfs*4	Global developmental delay, axial hypotonia, hypo/bradykinesia, dystonia, rigidity, gaze palsy, facial dyskinesia, dysphagia	5 years	Yes (ERT commenced at 20 months)	ABX withdrew within a month due to increase in tremor and axial instability
Patient 42 ^[44]	12 months	18 months	F	II-III	28% (RR < 30% compatible with GD)	NA	D448H/L422Pfs*4	Psychomotor delay, axial hypotonia, oculomotor apraxia by 1 year, hypokinesia and bradykinesia, action and rest tremor, retrocollis, lost the ability to walk with support by 4 years with hypokinetic-rigid syndrome, cogwheel rigidity at 5 years, dystonia, supranuclear saccadic horizontal gaze palsy, facial dyskinesias and dysphagia	5 years	Yes (ERT commenced at 20 months)	ABX withdrew within a month due to increase in tremor and axial instability

GluSph: Glucosylsphingosine; F: female; M: male; NA: not available; ABX: Ambroxol.

In a later study of four patients (14-20 years) with nGD and established myoclonic seizures and intellectual disability, combined therapy with high-dose ambroxol and ERT resulted in the arrest of neurological progression - including significantly decreased seizure frequency and improved neurocognitive function - only after the ambroxol dose was increased to 27 mg/kg/day. At this dose, GluSph levels normalized in all patients, and residual GCcase activity increased to approximately 9%-19% of the mean normal level, as evidenced by both *in vitro* and *in vivo* studies^[26]. The threshold enzyme activity required to prevent glucosylceramide accumulation has been suggested to be above 10%-15% of normal control^[45]. In addition, GCcase activity was reported to increase significantly in the brains of normal mice receiving 60 mg/kg/day ambroxol, which is equivalent to a human dose of 4.8 mg/kg/day^[46]. However, the precise increment in brain GCcase activity required to alleviate glucosylceramide and GluSph accumulation remains unknown. Importantly, ambroxol concentrations of 30 µmol/L or higher have been shown to inhibit certain GCcase mutants (R296Q and L483P)^[26].

Table 3. Table for conversion of legacy protein variant notation to the current HGVS standard nomenclature

Legacy protein variant	New HGVS nomenclature
84GG	L29fs
c.680_681delinsGG	N227R
D409H	D448H
E233D	E272D
F213I	F252I
F2123	F252I
G193W	G232W
G195E	G282E
G377S	G416S
H225Q	H294Q
H255Q	H294Q
L444P	L483P
N188S	N227S
R120W	R129W
R463H	R502H
V394L	V433L
Y211fs	V211fs
<i>RecNcil</i>	
A456P	A495P
L444P	L483P
V460V	V499=

A recent case detailed by Chu *et al.* described the use of ambroxol in a Taiwanese female neonate identified through newborn screening with markedly low GCase activity (0.47 $\mu\text{M}/\text{h}$; normal $> 1.8 \mu\text{M}/\text{h}$), significantly elevated chitotriosidase activity (577.62 nmol/mL WB/h; normal < 109.9), and L444P/RecNcil variants in *GBA*, a genotype predictive of GD2/3^[24]. ERT was introduced at five weeks of age due to thrombocytopenia, borderline splenomegaly, and elevated plasma GluSph, suggesting a severe phenotype. At six months, the patient displayed truncal rigidity and mild horizontal gaze palsy, prompting the initiation of high-dose ambroxol (30 mg/kg/day) at eight months. By 18 months, she showed delayed gross motor milestones but had not experienced developmental regression or seizures. Her clinical phenotype appeared relatively mild, supported by a modest elevation in baseline GluSph (117.8 ng/mL; normal < 3) and a mild response to ambroxol, as indicated by a further reduction in GluSph from 55.53 ng/mL post-ERT to 32.7 ng/mL after six months of ambroxol^[24].

In 2021, an investigator-initiated registry published an observational study on the safety and efficacy of ambroxol in patients with GD and *GBA*-associated Parkinson's disease (*GBA*-PD). Data were collected from 13 countries, comprising 41 patients with a median age of 17 years (range 1.5-74). Of these, 11 had GD1 (four also diagnosed with PD), 27 had nGD, and three were *GBA* mutation carriers with PD. The median treatment duration was 19 months (range 1-76), with a maximum daily ambroxol dose of 435 mg (range 75-1,485 mg). Clinical benefits, including neurological stability or improvement, increased physical activity, and reduced fatigue, were reported in 25 patients. Adverse effects were observed in 12 patients and included mild bowel discomfort, cough, allergic reactions, mild proteinuria, dizziness, and disease progression^[31]. A recent prospective study described the favorable clinical outcome of a Turkish female neonate with GD2 who presented with a collodion baby phenotype (ichthyosis). She received high-dose ambroxol monotherapy starting at four months of age. ERT was added one year later due to progressive hepatosplenomegaly. By three years of age, she demonstrated age-appropriate neurocognitive and motor

development. However, there was no clear benefit regarding visceral manifestations. Notably, cerebrospinal fluid GluSph levels decreased significantly compared to pre-treatment, although GluSph and chitotriosidase levels in the blood increased. *In vitro* studies of GCase activity in fibroblasts revealed a significant increase.

Including the three nGD patients described in this report and additional cases identified through a literature review from 2016 to 2024 [Table 2 and Table 3], a total of 45 patients have received ambroxol therapy. Treatment was discontinued in five patients due to adverse effects such as increased mucus production and cough. Additionally, two siblings with parkinsonian features experienced worsened tremors and axial instability. In two cases, reimbursement issues led to treatment discontinuation. Overall, approximately 81% of patients responded favorably to ambroxol chaperone therapy. In contrast, two GD2 patients managed at our center who did not receive ambroxol died at three and eight months of age, respectively^[47], consistent with previous reports of infant mortality in GD2 (median age of death: nine months)^[4].

Ascertainment of different GD subtypes has important therapeutic implications. Although current treatment options for nGD are limited due to the challenges of crossing the blood-brain barrier, ambroxol has emerged as a promising pharmacological chaperone treatment. In our case series, early detection accompanied by prompt intervention was crucial in modifying disease progression in Patient A with GD2, leading to reduced morbidity and mortality and improved clinical outcomes and overall quality of life. Early diagnosis of nGD also helps mitigate the prolonged diagnostic odyssey often faced by affected families, facilitates access to multidisciplinary supportive services, and supports informed reproductive decision making. These advantages have driven the development of newborn screening programs for GD, utilizing multiplex analytical techniques such as tandem mass spectrometry or digital microfluidics fluorimetry to measure GCase enzyme activity in DBS, with GluSph analysis serving as a second-tier test^[48]. GD is currently included in newborn screening panels in a few US states, including Illinois^[9], New York^[10], and Missouri^[49], though it has not yet been added to the Recommended Uniform Screening Panel (RUSP). Screening is also performed in Italy^[48], Taiwan^[50], Japan^[51], and mainland China^[52]. Determining the population-specific incidence of GD in Australia is essential to assess the potential benefit of implementing a national newborn screening program. Long-term outcome monitoring will require regular neurological examinations, radiological assessments, and formal developmental evaluations at defined intervals.

CONCLUSION

In summary, our case series expands the experience of Ambroxol therapy in nGD. To the best of our knowledge, we report the youngest GD2 patient who received disease-modifying treatment starting at just two weeks of age. This early intervention led to the complete resolution of respiratory compromise, hepatomegaly, and cytopenias, a significant reduction in GluSph, and a remarkably improved quality of life, with the patient achieving age-appropriate developmental milestones by 4 years of age - without the emergence of neurological symptoms or devastating life-limiting complications to date. In contrast, patients in our series who initiated Ambroxol therapy later in life experienced less substantial benefits in terms of neurologic disease stabilization or mitigation. These findings are consistent with the case published by Chu *et al.*, as well as several other studies, suggesting that Ambroxol therapy may delay or attenuate the progression of neurological symptoms in nGD^[24]. Therefore, early initiation of treatment appears to be critical for achieving optimal outcomes. The collective evidence on the utility of Ambroxol indicates that, when used in combination with ERT, it may offer a promising treatment for nGD. Although randomized clinical trials involving larger cohorts would be valuable to further assess outcomes in early-treated patients identified through sibling studies or newborn screening, we acknowledge that the likelihood of industry-sponsored clinical trials is low, given Ambroxol's low cost and limited commercial return. As a result, case reports like ours and those of other researchers play a vital role in advancing the understanding of this off-label use of Ambroxol chaperone therapy in GD.

DECLARATIONS

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Authors' contributions

Drafted the outline of the manuscript: Li K, Balasubramaniam S

Manuscript preparation: Vijayan K

Involved in the clinical management of the patients: Balasubramaniam S, Ismadi Z, Lichkus K, Gill D, Trivedi A, Curtin J

Performed GCase activity and GluSPH analysis on these patients' samples: Fuller M

All authors have read/critically revised the manuscript.

Availability of data and materials

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Conflicts of interest

Balasubramaniam S is a Junior Editorial Board member of *Journal of Translational Genetics and Genomics*, Balasubramaniam S was not involved in any steps of the editorial process, including reviewer selection, manuscript handling, or decision making, while the other authors have declared that they have no conflicts of interest.

Ethical approval and consent to participate

Ethics approval for publication of this case report was obtained from the Sydney Children's Hospital Network Human Research Ethics Committee (HREC project number: CCR2024/10). Informed consent for publication was obtained from all patients' parents.

Consent for publication

Not applicable.

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