The Clinical and Molecular Spectrum of GM1 Gangliosidosis

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Objective To evaluate the clinical presentation of patients with GM1 gangliosidosis and to determine whether specific clinical or biochemical signs could lead to a prompt diagnosis.

Study design We retrospectively analyzed clinical, biochemical, and genetic data of 22 patients with GM1 gangliosidosis from 5 metabolic centers in Germany and Austria.

Results Eight patients were classified as infantile, 11 as late-infantile, and 3 as juvenile form. Delay of diagnosis was 6 ± 2.6 months in the infantile, 2.6 ± 3.79 years in the late-infantile, and 14 ± 3.48 years in the juvenile form. Coarse facial features, cherry red spots, and visceromegaly occurred only in patients with the infantile form. Patients with the late-infantile and juvenile forms presented with variable neurologic symptoms. Seventeen patients presented with dystonia and 14 with dysphagia. Laboratory analysis revealed an increased ASAT concentration (13/20), chitotriosidase activity (12/15), and pathologic urinary oligosaccharides (10/19). Genotype analyses revealed 23 causative or likely causative mutations in 19 patients, 7 of them being novel variants. In the majority, a clear genotype–phenotype correlation was found.

Conclusions Diagnosis of GM1 gangliosidosis often is delayed, especially in patients with milder forms of the disease. GM1 gangliosidosis should be considered in patients with progressive neurodegeneration and spastic-dystonic movement disorders, even in the absence of visceral symptoms or cherry red spots. ASAT serum concentrations and chitotriosidase activity may be of value in screening for GM1 gangliosidosis. *(J Pediatr 2019;215:152-7)*.

GM1 gangliosidosis is a rare inborn error of metabolism caused by mutations in the galactosidase beta 1 (GLB1) gene leading to deficiency of the lysosomal enzyme β-galactosidase. This enzyme is involved in the degradation of glycoproteins, glycolipids, and keratan sulfate. The overall incidence of GM1 gangliosidosis is estimated to be 1:100 000-1:200 000 live births worldwide.[1] GM1 gangliosidosis, a neurodegenerative disorder with visceral and skeletal involvement, is classified into 3 clinical variants depending on age of onset and disease severity.[2,3] The infantile form (type I), with manifestation during the first year of life, is characterized by rapid progressive neurodegeneration, macular cherry-red spots, facial dysmorphism, skeletal dysplasia, hepatosplenomegaly, and early death. Type II GM1 gangliosidosis is divided into late infantile (type IIa) and juvenile (type IIb) forms. Patients with the late infantile form develop symptoms between first and third years of life, and patients with the juvenile form between third and tenth years of life. Both forms show a milder course and slower progression of the disease, presenting with seizures, spasticity, and dysostosis multiplex.[4-6] The adult form (type III), with manifestation after 10 years of life, is characterized by skeletal involvement, dystonia, gait, and/or speech disturbances and has been predominantly described in the Japanese population[1,2] (**Figure**).[3]

To date, more than 130 GLB1 mutations have been identified in patients with GM1 gangliosidosis.[7] There is no clear genotype–phenotype correlation. In this study, we analyzed clinical, biochemical, and genetic data to explore the natural history and the genotype–phenotype correlation in patients with GM1 gangliosidosis.

Methods

All patients with GM1 gangliosidosis who had been diagnosed within the last 20 years in 5 cooperating German and Austrian Metabolic University Centers were included in the study. A total of 22 patients (7 female, 15 male) with 3 pairs of siblings were included in the study. According to the previous established classification,[3] 8 patients were classified as infantile form (age at data evaluation 1.48 ± 3.97 years), 11 patients as late-infantile (age at data evaluation 7.97 ± 5.46 years), and 3 as juvenile (age at data evaluation 25.83 ± 2.67 years). Twenty-one patients were of European and 1 of Arabian

GLB1	Galactosidase beta 1

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origin; 6 patients with infantile form were deceased at the time of data collection.

All procedures were performed in accordance with the ethical standards of the national ethics committee and with the Helsinki Declaration of 1975, as revised in 2013.[8] As data were collected and analyzed only retrospectively, no approval was needed from our ethics committee. All data used in this study were anonymized. Informed consent was obtained from all patients and/or parents before being included in the study.

Data were collected retrospectively from patients' files and from patients and parents. Data collection included family and medical history, anthropometric data, and all routinely performed clinical, neurologic, ophthalmologic, and radiologic examinations.

Residual β-galactosidase activity was measured in leucocytes of 17 patients and in fibroblasts of 3 patients by using the artificial 4-methylumbelliferyl-b-galactopyranoside as substrate.[9] Aspartate-Aminotransferase (ASAT) serum concentration was reviewed in patients files. In 13 patients, chitotriosidase activity was measured in plasma, according to established methods.[10] Urinary oligosaccharides were analyzed by thin-layer chromatography in 19 patients.[11]

In 20 of 22 patients, molecular analysis of the GLB1 gene was performed from genomic DNA extracted from peripheral blood by Sanger sequencing according to standard methods.[12] Novel mutations were analyzed in silico (PolyPhen-2, Division of Genetics, Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts; SIFT, Genome Institute of Singapore [A*STAR], Singapore, Singapore; MutationTaster, Berlin Institute of Health, Charité, Berlin, Germany; Alamut, Interactive Biosoftware, Rouen, France; and Human Splicing Finder, Bioinformatics & Genetics Team, L'Université d'Aix-Marseille, Marseille, France) to predict the effect on the protein level. All detected mutations were checked against publicly available reference datasets, ie, dbSNP150, 1000 Genomes Project, Exome Variant Server, gnomAD population database, VarSome database, Online Mendelian Inheritance in Man, Human Gene Mutation Database, and ClinVar database. Classification of pathogenicity was carried out according to the American College of Medical Genetics/American College of Pathology guidelines.[13]

Statistical Analyses

Data were analyzed by Excel in Microsoft Office Home and Business 2013 (Version 15.05101.1002; Microsoft, Redmond, Washington). Due to the small number of patients, data were evaluated mainly in descriptive manner.

Results

Patients with the infantile form presented with muscular hypotonia at age 0.21 ± 0.22 years. Patients with the late-infantile form presented with variable motor and non-motor neurologic symptoms, ie, pyramidal signs, dystonia, dysarthria, and cognitive decline, at age 1.92 ± 0.54 years. Patients with the juvenile form developed gait abnormalities accompanied by hip pain at age 3.00 ± 0.47 years (**Table I**). Time between first symptoms and diagnosis was 0.56 ± 0.23 years in patients with the infantile, 2.6 ± 3.79 years in patients with the late-infantile, and 14 ± 3.48 years in patients with the juvenile form.

Visceromegaly, ie, cardiomyopathy and hepatosplenomegaly, was a characteristic symptom of patients with infantile form (**Table II**). Only 1 patient with late infantile and none with juvenile form showed visceromegaly. All patients with infantile form presented typical facial dysmorphism and none of those with late-infantile or juvenile form. At time of diagnosis, 8 of 22 patients revealed short stature, 7 of 22 failure to thrive, 5 of 21 microcephaly, and 6 of 21

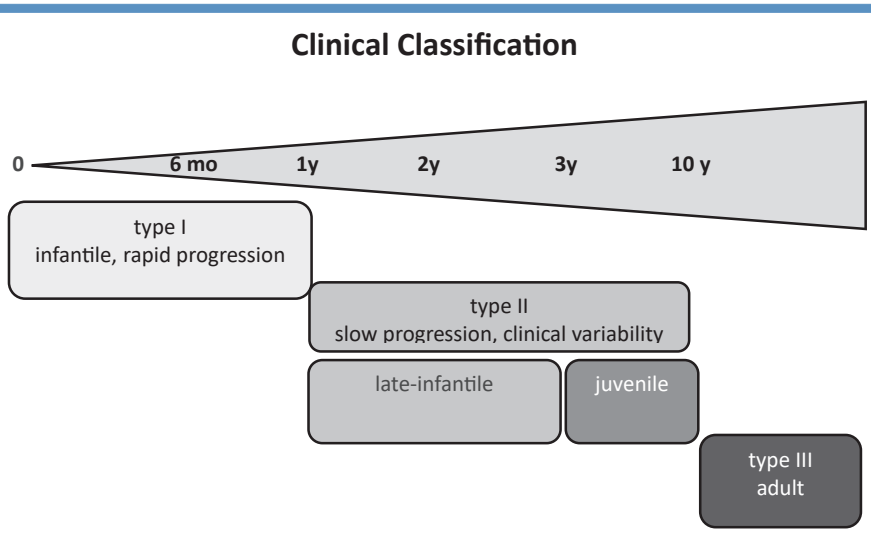

Figure. Classification of GM1 gangliosidosis according to occurrence of first symptom.[3]

Table I. Clinical and radiologic findings at manifestation and in the course of the disease

						Visceral symptoms		Skeletal symptoms					Neurological symptoms							
ID	Phenotype	Age, y	Age at first symptom, y	Age at diagnosis, y	Symptom(s) at time of manifestation	Cardiomyopathy	Hepatosplenomegaly	Coarse face	Macrocephaly	Microcephaly	Spinal deformity	Dysostosis multiplex	Cognitive decline	Seizures	Muscular hypotonia	Pyramidal signs	Dystonia	Ataxia	Dysphagia	Red macula spot
1	Infantile	1.28*	0.08	3.50	Hepatosplenomegaly	x	x	x	x		x	x	x		x	x			x	
2	Infantile	1.28*	0.25	6.00	Muscular hypotonia		x	x			x		x	x	x	x	x			x
3	Infantile	na	0.24	11.87	Muscular hypotonia			x	x		x	x	x		x	x	x	x		
4	Infantile	1.98*	0.17	8.73	Coarse face; muscular hypotonia	x		x			x	na	x	x	x	x			x	
5	Infantile	1.52*	0.08	7.87	Muscular hypotonia			x			x	x	x	x	x	x			x	x
6	Infantile	1.35*	0.50	10.80	Pulmonary infection	x	x	x		x			x	x	x	x	x			x
7	Infantile	1.44*	0.00	7.10	Edema of hands, feet, testes; muscular hypotonia; deformity of vertebral bodies	x	x	x			x	x	x	x	x	x	x		x	
8	Infantile	8.34	1.16	1.51	Gait abnormality; psychomotor deficit	x	x	x		x	x	x	x		x	x	x		x	
9	Late-infantile	7.86	1.50	3.23	Psychomotor deficit						x	x	x	x	x	x	x		x	
10	Late-infantile	12.71	1.50	4.29	Pes equinus	x					x		x	x		x	x	x	x	
11	Late-infantile	10.09	1.92	2.01	Pes equinus				x		x		x	x		x	x	x	x	
12	Late-infantile	17.54	2.00	15.96	Dysarthria						x		x		x	x	x		x	
13	Late-infantile	7.97	2.00	6.39	Dysarthria; gait abnormality						x		x		x	x	x		x	
14	Late-infantile	4.96	0.83	2.65	Dysarthria							na	x		x	x	x	x	x	
15	Late-infantile	2.76	1.75	0.44	Psychomotor deficit				x			x	x	x		x	x	x	x	
16	Late-infantile	20.87	2.00	6.00	Dysarthria						x	x	x	x	x	x	x			
17	Late-infantile	5.82	2.92	5.25	Gait abnormality				x			na	x	x	x	x	x			
18	Late-infantile	8.54	2.42*	9.90	Psychomotor deficit					x			x		x	x	x	x	x	
19	Late-infantile	3.32	1.24	3.85	Psychomotor deficit								x	x	x	x	x	x	x	
20	Juvenile	25.83	3.00	18.20	Dystonia; psychomotor deficit					x	x	x	x		x		x			
21	Juvenile	20.73	4.00	11.31	Psychomotor deficit						x	x	x		x			x		
22	Juvenile	26.81	3.00	17.05	Psychomotor deficit						x	x	x	x						
Percentage of symptoms in total						27%	23%	36%	23%	18%	73%	58%	100%	59%	77%	86%	77%	36%	64%	14%

Siblings: patients 10,11; patients 12,13; and patients 14,15. Patient 3 lost to follow-up.
*Age at death.

Table II. Symptoms and biochemical signs in 3 clinical forms of GM1 gangliosidosis

Symptoms and signs	Infantile form	Late-infantile form	Juvenile form
Clinical symptoms			
Coarse facial features	+	−	−
Cherry red macula spot	−/+	−	−
Cardiomyopathy	−/+	−/+	−
Hepatosplenomegaly	−/+	−	−
Cognitive decline	+	+	+
Dystonia	−/+	−/+	−/+
Ataxia	−/+	−/+	−/+
Pyramidal signs	+	+	−
Laboratory signs			
Increased oligosaccharides	+	−/+	−/+
Increased ASAT	−/+	−/+	−
Increased chitotriosidase activity	>1000	>100–1000	na

na, not applicable.

macrocephaly. The majority of all patients with all different forms of GM1 gangliosidosis revealed radiologic signs of dysostosis multiplex (Table I). Spinal deformity was a frequent symptom in the majority of all forms of GM1 gangliosidosis and presented most frequently as scoliosis and thoracolumbal gibbus formation.

Cognitive decline and muscular hypotonia were the most frequent neurologic symptoms. Of 12 patients with seizures, 5 patients with infantile and 1 patient with late-infantile form developed seizures within their first year of life. All patients with infantile and late-infantile form, but none of those with juvenile form, presented pyramidal signs, defined as at least pathologic exaggerated reflexes. Dystonia was found in 5 of 8 patients with infantile form in all 11 patients with late-infantile form and in 1 of 3 patients with juvenile form. Gait ataxia was seen in one-half of the patients with late-infantile form and 1 patient with juvenile form. Dysphagia was reported in 5 of 8 patients with infantile form, 8 of 11 patients with late-infantile form, and 1 patient with juvenile form. Cherry-red spots were detected only in 3 patients with infantile form but not in any other form of GM1 gangliosidosis. Cranial magnetic resonance imaging revealed pathologic signs in 16 of 18 patients among all 3 subtypes. Pathologic findings included cortical and cerebellar atrophy, leukoencephalopathy, and dilated Virchow Robinson spaces.

Serum chitotriosidase activity was increased in 9 of 13 patients (Table III; available at www.jpeds.com). Patients with the infantile form revealed significant greater chitotriosidase activity than those with the late-infantile form (1888 ± 758 nmol/mL/h vs 182 ± 291 nmol/mL/h); however, 1 patient with juvenile form showed an activity of 1393 nmol/ml/h. Analyses of urinary oligosaccharides revealed a GM1 gangliosidosis typical pathologic pattern in 7 of 7 patients with infantile, in 2 of 9 with late-infantile, and in 3 of 3 with juvenile form (Table III). Surprisingly, only patients with late-infantile form had a normal pattern of urinary oligosaccharides. ASAT serum concentrations were elevated in 13 of 20 patients, both with infantile and late-infantile forms.

Our data showed no correlation between residual β-galactosidase activity and the clinical phenotype and/or genotype. Genotype analyses revealed 23 different causative or likely causative mutations in 19 patients: 16 previously described and 7 yet-unreported variants. In overall, 14 missense, 4 frameshift, 2 nonsense, and 3 splice-site variants were found (Table III). Nine patients with late-infantile form (including 2 pairs of siblings) were compound heterozygous for a deleterious allele [p.(R457Q), p.(A301V), c.75+2dupT] and a milder variant [p.(L155R), p.(R201H), p.(T82M)] or a novel missense variant [p.(R595P), p.(R419W)].

Seven yet-unreported variants (3 frameshift, 2 splice-site, and 2 missense) were detected in 8 patients. Patient 5 with infantile form was compound heterozygous for 2 novel frameshift mutations: c.1657dupA, p.(M553Nfs*32) and c.1841dupA, p.(N614Kfs*16), both classified as likely pathogenic. Patient 8 carries the known c.75+2dupT pathogenic mutation in trans to a synonymous substitution c.1461A>G. We could show by reverse transcription polymerase chain reaction analysis that c.1461A>G introduces a novel splice site leading to the deletion of 23 bp (c.1457-1479del), resulting in a frameshift with a premature stop codon (p.Gly486GlyfsX4, class 4 mutation) and a 188 amino acids–shortened mutated protein. Siblings 12 and 13, with the late-infantile form, carried 1 known mutation, previously described in an adult form, and a second novel splice-site mutation c.1233+1G>T, classified as likely pathogenic. In the siblings 14 and 15, with the late-infantile form, the yet-unreported missense variant p.(R595P) of uncertain significance occurred in combination with a deleterious missense mutation previously described in infantile patients. Patient 19 was compound heterozygous for p.(T82M), previously shown to be associated with an adult form, and a yet-unreported missense variant p.(R419W), classified as likely pathogenic. The novel frameshift variant c.287delA [p.(Y96Sfs*25), class 4] in combination with a milder mutation p.(R201H) was identified in patient 22, with a juvenile form.

Discussion

This multicenter study presents clinical and molecular data of a large cohort of patients with different forms of GM1 gangliosidosis. These data reveal that diagnosis of GM1 gangliosidosis is delayed, especially in patients with disease onset after the first year of life. Poor awareness of this rare disease and the often-unspecific variable clinical symptoms of GM1 gangliosidosis make diagnosis difficult and may account for the large delay between manifestation and diagnosis.

Our data reveal that a few clinical symptoms are characteristic for the different forms of GM1 gangliosidosis and may be considered for differential diagnosis. Visceral symptoms, ie, hepatosplenomegaly and cardiomyopathy, are a leading symptom in patients with the infantile form, but not in those with a milder form of the disease, ie, in patients with late-infantile or juvenile form. In addition, facial dysmorphism was shown to be a typical symptom in all patients with

infantile form, thus being previously reported with different frequency.[1,14]

Cognitive decline was present in all forms of GM1 gangliosidosis of this study, even in those with juvenile form. This is in contrast to previous studies reporting cognitive decline only in infantile and late-infantile form but not in patients with juvenile form.[1] Our data support previous data that muscular hypotonia and seizures are a common feature in all forms of GM1 gangliosidosis.[1,15] However, they seem to be more frequent in patients with infantile and late-infantile forms. In addition, pyramidal signs were a characteristic symptom in patients with infantile and late-infantile, but not in those with juvenile form. Our data reveal that dystonia and ataxia are present in all forms of GM1 gangliosidosis, especially in the late-infantile form. This confirms the data reported in patients from South Iran.[5] Dysphagia was a leading symptom in >70% of the patients with infantile and late-infantile forms, which again is greater than previously reported and reflects underdiagnosis of dysphagia as an important symptom in GM1 gangliosidosis.[16] This is of clinical importance, as dysphagia may result in malnutrition and consequently in failure to thrive, which was diagnosed in 64% of our patients. This underlines the prompt diagnosis of dysphagia to initiate early gastric tube feeding of these patients for a better survival of the patients. Further clinical symptoms in all forms of GM1 gangliosidosis were macrocephaly and microcephaly, indicating a greater frequency than earlier reported (29% vs 3%-19%, 24% vs 3%-5%, respectively).[15,16]

Cherry red macula spots were detected only in 3 of 8 patients with infantile form and in none of those with late-infantile and juvenile forms. In previous studies, 40%-60% of the patients with infantile GM1 gangliosidosis presented cherry red spots.[1,14,15,17] This is of great importance for differential diagnosis of GM1 gangliosidosis, as cherry red macula spot is not one of the most frequent leading clinical symptoms.

Patients with the infantile form had a 10-fold greater plasma chitotriosidase activity than those with late-infantile form, indicating a correlation between level of chitotriosidase activity and clinical phenotype. However, 1 patient with the juvenile form revealed a high chitotriosidase activity, which might be caused by an additional inflammation process.[18] Thus, the differential diagnosis of enhanced chitotriosidase activity should include GM1 gangliosidosis.[19]

Pathologic pattern of urinary oligosaccharides were detected in all patients with infantile form, which is in line with previous reports.[20,21] In contrast to patients with juvenile form, most patients with late-infantile form of GM1 gangliosidosis revealed normal oligosaccharide pattern. Thus, urinary oligosaccharide analyses seem to be not an adequate screening tool for milder forms of GM1 gangliosidosis.

Elevated ASAT serum concentrations have been reported in patients with GM1 gangliosidosis.[6,14] We confirmed these data with elevated ASAT concentrations in 13 of 20 patients, which allow consideration of analysis of serum ASAT as an additional screening marker for GM1 gangliosidosis.

No clear genotype–phenotype correlation has been reported yet in patients with GM1 gangliosidosis. However, it is known that certain GLB1 mutations may influence folding and substrate binding of the mutant enzyme and consequently the residual enzyme activity,[1,22] thus resulting in different clinical phenotypes. In our cohort, a correlation between genotype and phenotype was shown for the majority of different forms of GM1 gangliosidosis. Five of 7 patients with infantile form carried 2 homozygous or in 1 case compound heterozygous severe mutations reported before in infantile forms. In addition, 1 patient with infantile form was compound heterozygous for the 2 novel frameshift variants c.1657dupA and c.1841dupA, both classified as likely pathogenic mutations, resulting in very low residual enzyme activity and, thus, accounting for severe form of the disease. In Patient 8, the combined impact of 2 splice-site variants (1 associated with an infantile form in homozygous state and 1 novel splice-site variant) led to the infantile course of the disease. A similar combination of deleterious frameshift and milder missense mutations were described recently in Chinese patients with late-infantile GM1-gangliosidosis.[14] In 5 patients with late-infantile form, novel variants revealing a clear genotype–phenotype correlation were identified. The novel splice-site mutation c.1233+1G>T in trans with the known milder missense mutation p.L155R[24] accounted for the milder late-infantile course in siblings 12 and 13. In patient 15, the novel missense variant p.(R595P) in association with a known deleterious missense mutation, previously described in infantile patients,[9,25] led to a milder manifestation with a late-infantile form. Lee et al described a novel missense mutation in the same domain p.(Y591H) of the GLB1 gen in a Korean patient with late-infantile GM1-gangliosidosis.[6] In patient 19, the milder form of the disease seems to be caused by the combination of a previously described missense mutation in association with adult form p.(T82M)[26] and a novel missense variant p.(R419W) located in a moderately conserved region. In patient 22, with juvenile form, the novel frameshift variant c.287delA, more likely to be associated with a severe form of the disease, was identified in trans with a milder mutation p.(R201H) previously detected in adult forms.[1] It accounted for ~60% residual enzyme activity[14] and may have accounted for the milder form of the disease. In the patient presenting as a juvenile form with dystonia and psychomotor regression, the p.(R201H) mutation was found in trans with a known nonsense mutation. Karimzadeh et al found a homozygous founder mutation in the same codon (R201C) in 3 families from the Southwest part of Iran with juvenile GM1-gangliosidosis presenting with ataxia, gait disturbances, dystonia, and general developmental regression,[5] underlining the effect of this specific conserved amino acid in the development of a juvenile form. Our data show that

by intensive analyses a genotype–phenotype correlation can be found in the majority of patients with GM1 gangliosidosis. These findings will help future genetic counseling of affected families, prognosis of the outcome of affected patients, and decisions on treatment.

In conclusion, GM1 gangliosidosis must be considered in all patients presenting with progressive neurodegeneration and spastic-dystonic movement disorders of unknown origin, even in the absence of facial dysmorphism, cherry red spots, or visceral symptoms. ■

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Table III. Identified GLB1 mutations in the patients and additional biochemical data

Patient ID	Clinical type of GM1	Zygosity	Variant(s) identified in the GLB1 (OMIM *611458) gene Transcript: NM_000404	Classification of the variant*	AA conservation	Previous description of the mutation	Atypical oligosaccharide Pattern in urine	Chitotriosidase activity (5-100 mU/mL)	ASAT
1	Infantile form	hom	c.176G>A p.(R59H)	Class 5 HGMD: CM990696 dbSNP: rs72555392 (pathogenic)	highly conserved AA	Rapid progressive infantile form with cardiac involvement[27]	Yes	1.130	1.1× ULN
2	Infantile form	hom	c.1577dupG p.(G526Gfs*5)	Class 5 HGMD: CI992013 dbSNP: rs794729217 (pathogenic)		Severe infantile form[12]	Yes	na	Normal
3	Infantile form	comp het	c.483G>A p.W161* mat + c.622C>T p.(R208C) pat	Class 5 HGMD: CM105071 (pathogenic) Class 5 HGMD: CM930342 dbSNP: rs72555366 ClinVar: pathogenic	Highly conserved AA	Infantile form[9] Infantile form[23]	na	na	Normal
4	Infantile form	hom	c.1471G>T p.(D491Y)	Class 5 HGMD: CM070943 and CM990699 (pathogenic)	Highly conserved AA	Infantile form[24]	Yes	2.646	na
5	Infantile form	comp het	c.1657dupA p.(M553Nfs*32) + c.1841dupA p.(N614Kfs*16)	Class 4 (likely pathogenic) Class 4 (likely pathogenic)		Novel frameshift variant Novel frameshift variant	Yes	na	2× ULN
6	Infantile form	na	na	na	na	na	Yes	na	Normal
7	Infantile form	hom	c.808T>G p.(Y270D)	Class 5 HGMD: CM012603 ClinVar: pathogenic, infantile form	highly conserved AA	juvenile form in heterozygous state[28]	Yes	na	2× ULN
8	Infantile form	comp het	c.75+2dupT p.? + c.1461A>G cDNA level: c.1457-1479del p.Gly486GlyfsX4	Class 5 ClinVar: pathogenic, infantile form Class 4 experimental evidence for novel splice site and deletion of 23 bp		Infantile form in homozygous state and adult form in compound heterozygous state[26] Novel splice-site variant	Yes	Increased	4× ULN
9	Late infantile form	hom	c.1733A>G p.(K578R)	Class 5 HGMD CM930343 dbSNP: rs371582179 ClinVar: pathogenic, late infantile form	Highly conserved AA	Infantile form in heterozygous state[23]	No	1.013	2× ULN
10	Late-infantile form	comp het	c.464T>G p.(L155R) pat + c.1370G>A p.(R457Q) mat	Class 5 HGMD: CM070944 dbSNP: rs376710410 (pathogenic) ClinVar: pathogenic Class 5 HGMD: CM910187 dbSNP: rs28934886 (pathogenic) ClinVar: pathogenic, adult form	Highly conserved AA Highly conserved AA	Adult form in homozygous state[24] Infantile form,[2] Adult form in heterozygous state[29]	No	182	2× ULN

(*continued*)

Table III. Continued

Patient ID	Clinical type of GM1	Zygosity	Variant(s) identified in the GLB1 (OMIM *611458) gene Transcript: NM_000404	Classification of the variant*	AA conservation	Previous description of the mutation	Atypical oligosaccharide Pattern in urine	Chitotriosidase activity (5-100 mU/mL)	ASAT
11	Late-infantile form	comp het	c.464T>G p.(L155R) pat + c.1370G>A p.(R457Q) mat	Class 5 HGMD: CM070944 dbSNP: rs376710410 (pathogenic) ClinVar: pathogenic Class 5 HGMD: CM910187 dbSNP: rs28934886 (pathogenic) ClinVar: pathogenic, adult form	Highly conserved AA highly conserved AA	Adult form in homozygous state[24] Infantile form,[2] adult form in heterozygous state[29]	No	277	1.2× ULN
12	Late-infantile form	comp het	c.464T>G p.(L155R) mat + c.1233+1G>T p.? pat	Class 5 HGMD: CM070944 dbSNP: rs376710410 (pathogenic) ClinVar: pathogenic Class 4 (likely pathogenic)	Highly conserved	Adult form in homozygous state[24] Novel splice site variant	No	Null-mutation	1.6× ULN
13	Late-infantile form	comp het	c.464T>G p.(L155R) mat + c.1233+1G>T p.? pat	Class 5 HGMD: CM070944 dbSNP: rs376710410 (pathogenic) ClinVar: pathogenic Class 4 (likely pathogenic)	Highly conserved	Adult form in homozygous state[24] Novel splice-site variant	Yes	Increased	1.3× ULN
14	Late-infantile form	comp het	c.902C>T p.(A301V) mat + c.1784G>C p.(R595P) pat	Class 5 HGMD: CM105083, CS064415 ClinVar: pathogenic Class 3 (uncertain significance) Mutation Taster: Polymorphism SIFT: tolerated PolyPhen2: possibly damaging	moderately conserved	Infantile form[9,25] Possible novel missense mutation	No	165	5× ULN
15	Late-infantile form	comp het	c.902C>T p.(A301V) mat + c.1784G>C p.(R595P) pat	Class 5 HGMD: CM105083, CS064415 ClinVar: pathogenic Class 3 (uncertain significance) Mutation Taster: Polymorphism SIFT: tolerated PolyPhen2: possibly damaging		Infantile form[9,25] Possible novel Missense mutation	No	118	Normal
16	Late-infantile form	comp het	c.602G>A p.(R201H) + c.841C>T p.(H281Y)	Class 5 HGMD: CM972865 dbSNP: rs189115557 ClinVar: pathogenic Class 5 HGMD: CM012604 dbSNP: rs745386663 ClinVar: pathogenic	Highly conserved AA Highly conserved AA	Juvenile and adult form in compound heterozygous state[29,30] Juvenile form in compound heterozygous state[28]	Yes	na	1.4× ULN
17	Late-infantile form	na	na	na	na	na	No	176	2× ULN

(*continued*)

Table III. Continued

Patient ID	Clinical type of GM1	Zygosity	Variant(s) identified in the GLB1 (OMIM *611458) gene Transcript: NM_000404	Classification of the variant*	AA conservation	Previous description of the mutation	Atypical oligosaccharide Pattern in urine	Chitotriosidase activity (5-100 mU/mL)	ASAT
18	Late-infantile form	comp het	c.602G>A p.(R201H) + c.202C>T p.(R68W)	Class 5 HGMD: CM972865 dbSNP: rs189115557 ClinVar: pathogenic Class 5 HGMD: CM031192 dbSNP: rs72555370 ClinVar: pathogenic, juvenile form	Highly conserved AA Highly conserved AA	Juvenile and adult form in compound heterozygous state[29,30] Late-infantile form in compound heterozygote state[3]	na	Increased	normal
19	Late-infantile form	comp het	c.245C>T p.(T82M) mat + c.1255C>T p.(R419W) pat	Class 5 HGMD CM940869 dbSNP: rs72555393 ClinVar: pathogenic, adult form Class 4 dbSNP: rs747709527 (likely pathogenic)	Moderately conserved	Adult form in compound heterozygous state[26] Novel missense variant	na	236	1.2× ULN
20	Juvenile form	comp het	c.1369C>T p.R457* + c.602G>A p.(R201H)	Class 5 HGMD: CM910188 dbSNP: rs72555359 ClinVar: pathogenic, infantile form Class 5 HGMD: CM972865 dbSNP: rs189115557 ClinVar: pathogenic	Highly conserved AA	Infantile form[31] Juvenile and adult form in compound heterozygous state[29,30]	Yes	1.393	Normal
21	Juvenile form	na	na	na	na	na	Yes	na	Normal
22	Juvenile form	comp het	c.602G>A p.(R201H) + c.287delA p.(Y96Sfs*25)	Class 5 HGMD: CM972865 dbSNP: rs189115557 ClinVar: pathogenic Class 4: (likely pathogenic)	Highly conserved AA	Juvenile and adult form in compound heterozygous state[29,30] Novel frameshift variant	Yes	na	na

comp het, compound heterozygous; *hom*, homozygous; *mat*, maternal; *pat*, paternal; *ULN*, upper limit of normal.
*Classification of the variants according to Richards et al.[13] All detected variants were checked against publicly available reference datasets.