Disentangling molecular and clinical stratification patterns in beta-galactosidase deficiency

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ABSTRACT

Introduction: This study aims to define the phenotypic and molecular spectrum of the two clinical forms of β-galactosidase (b-GAL) deficiency, GM1-gangliosidosis and Mucopolysaccharidosis IVB (Morquio disease type B, MPSIVB).

Methods: Clinical and genetic data of 52 probands, 47 GM1-gangliosidosis and 5 MPSIVB patients, were analyzed.

Results: The clinical presentations in GM1-gangliosidosis patients are consistent with a phenotypic continuum ranging from a severe antenatal form with hydrops fetalis to an adult form with an extrapyramidal syndrome. Molecular studies evidenced 47 variants located throughout the sequence of the *GLB1* gene, in all exons except 7, 11 and 12. Eighteen novel variants (15 substitutions and 3 deletions) were identified. Several variants were linked specifically to early-onset GM1-gangliosidosis, late-onset GM1-gangliosidosis or MPSIVB phenotypes. This integrative molecular and clinical stratification suggests a variant-driven patient assignment to a given clinical and severity group.

Conclusion: This study reports one of the largest series of b-GAL deficiency with an integrative patient stratification combining molecular and clinical features. This work contributes to expand the community knowledge regarding the molecular and clinical landscapes of b-GAL deficiency for a better patient management.

INTRODUCTION

The acid β-galactosidase enzyme (b-GAL, EC 3.2.1.23) hydrolyzes the terminal β-galactosyl residues from GM1-gangliosides and β-galactosyl-containing molecules within the lysosome. β-galactosidase deficiency is a rare autosomal recessive disorder characterized by the accumulation of GM1-gangliosides and the mucopolysaccharide keratan sulfate (KS). [1]

β-galactosidase deficiency pathophysiology and its subsequent phenotypic expressions are multifactorial and highly complex. This complexity may be partly related to (i) the alteration of both *GLB1* gene products, b-GAL and elastin-binding protein (EBP), (ii) the pivotal roles of non-degraded products. Gangliosides are constituents of plasma membranes and have functional roles in signaling and cellular processes [2] while KS is linked to extracellular matrix proteins to form proteoglycans.

b-GAL deficiency includes two phenotypically distinct lysosomal disorders. [3] Mucopolysaccharidosis type IVB (MPSIVB, Morquio disease type B, OMIM#253010) is characterized by marked skeletal abnormalities, increased urinary excretion of KS with no signs of storage in neuronal tissues. [3] GM1-gangliosidosis (OMIM: #203500, #203600, #203650) clinical settings involve a progressive neurodegeneration due to the massive storage of GM1-gangliosides within the central nervous system.[4] Initially, GM1-gangliosidosis has been divided clinically into three groups of increasing severity.[1] However, the clinical course must be regarded as a continuum, ranging from the most severe antenatal cases to the adult form.

The present work aims to report the molecular and clinical landscapes of one of the largest b-GAL deficiency series described to date spanning a broad range of clinical severity and forms with an integrative patients' stratification combining molecular and clinical features.

PATIENTS AND METHODS

Patients

Fifty-two patients with b-GAL deficiency have been included in this descriptive study. Molecular analyses were performed in Rouen university hospital, France from 2006 to 2020. All patients included in this study were seen at the outpatient clinic by a pediatrician, a metabolician or a clinical geneticist. A questionnaire, requesting information about pregnancy, past medical history, cognitive/behavioral, sensorial and motor milestones, and first clinical, biochemical and radiological features and EEG analyses, was filled out by caregivers. For the clinical description, the terms of the Human Phenotype Ontology (HPO https://hpo.jax.org/app/) have been used.

Biochemical diagnosis

Clinical diagnosis was confirmed by the assessment of residual β-galactosidase activity in (i) cultured amniocytes or leukocytes using the artificial substrate, 4-methylumbelliferyl β-galactopyranoside mainly in 4 laboratories in France (Rouen, Toulouse, Lyon and Grenoble University Hospitals) (ii) or on dried blood spot using a mass spectrometry method (Rouen University Hopital). The assessment of neuraminidase activity was carried out for all the patients and allowed to confirm the isolated β-galactosidase deficiency.

Genetic analysis

Genomic DNA of the 52 patients and their non-affected parents was extracted from peripheral blood using QIAamp DNA Blood Mini Kit® Qiagen or the QuickGene-610L platform (Kurabo Biomedical, FujiFilm). *GLB1* gene was sequenced using Sanger or Next Generation Sequencing (NGS) methods.

For Sanger sequencing, PCR reaction was carried out in 1X Thermo Scientific Buffer IV: 75mM Tris-HCL pH 8,8, 20mM $(NH_4)_2SO_4$, 0,01% Tween 20, 1,5mM $MgCl_2$, 100µM each of dNTPs, 1,25U/µl Taq polymerase, 0,6 µM of each primer (primer sequences are available upon request). Touchdown PCR

consisted of one cycle of 95°C for 5 min for the initial denaturation step followed by 12 cycles of denaturation at 95°C for 25 s, varying annealing (60-48°) for 25 s, and extension at 72°C. Then, 35 cycles were performed as follows: denaturation at 95° for 25s, annealing at 48° for 25 s and extension at 72°C for 25 s. PCR was terminated after a final cycle at 72°C for 5 min. Direct DNA fragments sequencing was performed with an ABI prism big dye Terminator cycle Sequencing Ready Reaction Kit (PE Applied Biosystem and ABI model 3130xl Genetic Analyzer, CA, USA). Patient genomic sequence comparison with the reference sequence was done using the Variant Reporter software (Applied Biosystem).

Since 2016, a targeted capture sequencing panel including 52 lysosomal has been implemented on an Illumina® platform (San Diego, CA, USA). The panel was designed using the Agilent SureDesign Software (Agilent Technologies Inc., Santa Clara, CA, USA). For all genes, the coding region and ± 50 bp within the flanking intronic sequences (296 kb, 506 regions) were targeted. Library construction was performed using SureSelect QXT (Agilent Technologies Inc., Santa Clara, CA, USA) and sequencing was performed on MiSeq or NextSeq instruments (Illumina®, CA, USA) using 2×150 bp paired-end sequencing. The panel design and the sequencing protocol are available upon request. Data analysis has been previously described [5]. Briefly, bioinformatics pipeline including CASAVA suite v1.8 (Illumina®, CA, USA) and BWA-GATK 2.2.5. (Genome Analysis ToolKit, Broad Institute, Cambridge, MA, USA) has been used for mapping and variant calling, Alamut Batch (Interactive BioSoftware, Rouen, France) for variant annotation, and CanDiD database for prioritizing and filtering variants of interest. The CANOES algorithm (CNVs with an Arbitrary Number Of Exome Samples) allowed the detection of copy number variants (CNVs) such as deletions or duplications. Alamut software (Interactive Biosoftware Rouen, France) was used to mine the identified variations. The described variations were named according to the current nomenclature recommendations (http://www.hgvs.org/mutnomen) using the NM_000404.3 sequence.

Allele frequency analysis

The frequency in human population of the novel variants was evaluated using Genome Aggregation Database browser (gnomAD, http://gnomad.broadinstitute.org/; accessed in April 2020).

Computational analysis of the functional impact of missense variants

As described previously [5], the pathogenicity of missense variants was evaluated using *in silico* methods including Align GVGD, SIFT, PolyPhen2, MutationTaster and M-CAP (Mendelian Clinically Applicable Pathogenicity). M-CAP combines the pathogenicity scores of several algorithms including SIFT, Polyphen-2 and CADD to classify typical exome/genome rare (<1%) missense variant.

Bioinformatics predictions of splicing variants

The impact of variants on splicing was evaluated using *in silico* tool MaxEntScan interrogated by Alamut® (Interactive Biosoftware) and the SPiP (Splicing Prediction Pipeline) tool.

Variant classification

The novel variants were graded using the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) classification system (https://www.amp.org/clinical-practice/practice-guidelines/).

Ethics Statement

The study was performed according French ethical law (NOR: AFSP1313547A) regarding genetic investigations for diagnosis purposes. Written informed consents were obtained from the parents when the patient is under 18 or from the adult patient in order to perform any investigation related to their pathology.

RESULTS

Clinical findings

Fifty-two patients with b-GAL deficiency were included in this study (**figure 1A**, **Supplementary tables S1 and S2**). Forty-seven patients presented with GM1-gangliosidosis, the male/female ratio was at 19/28. Five female patients exhibited a phenotype consistent with MPSIVB (**figure 1A**, **Supplementary**

tables **S1 and S2**). Comprehensive clinical data were obtained for 43 out of 52 patients (**Supplementary tables S1 and S2**). Consanguinity was noted in 27 probands out of 52.

Among the GM1-gangliosidosis patients, the phenotypic continuum was as follows: 36 patients with an early-onset phenotype (age of first symptoms: before age of 2 years), 11 late-onset cases (age of first symptoms: 2 – 25 years) (**Supplementary tables S1 and S2**).

As illustrated in **figures 1C** and **1D,** a broad range of clinical features is identified in GM1 patients with a decreasing order of frequency as follows: neurological signs, musculoskeletal abnormalities, gastrointestinal, visual impairment, dysmorphic features, respiratory and cardiac signs (**Supplementary table S3**). Ten index cases had an antenatal presentation with a hydrops fetalis (**Supplementary tables S1 and S2**).

The female patient with the adult form (**P10**, **Supplementary table S1**) consulted at the age of 25 years for gait disturbances and muscle cramps. On examination, she presented with a left side extrapyramidal syndrome with dystonic movements. Of note, the patient's history was marked by bone dysplasia complicated by osteonecrosis of the femoral heads since childhood.

MPSIVB patients presented mainly with musculoskeletal abnormalities and facial dysmorphism with an age of diagnosis between 4 and 18 years (**figure 1C** and **1D, Supplementary table S1 and S2**).

Molecular findings

From 2006 to 2020, a total of 52 patients with b-GAL deficiency have been investigated. b-GAL deficiency diagnosis was demonstrated by the reduction or the absence of β-galactosidase enzyme activity in fibroblasts, DBS and/or leukocytes in all included patients; 42 out of 52 b-GAL activities are reported in **Supplementary table S1**.

Two mutated alleles were identified in all patients (**Supplementary tables S1, S4 and S5**) and the unaffected parents were heterozygous for one allele. Twenty-seven patients were born to consanguineous parents and presented with homozygous variants (**Supplementary tables S1, S4 and**

S5). Forty-seven variations were identified in *GLB1* gene sequence (NM_000404.3). Twenty-nine variants have been previously published[6-19] and 18 are novel (**Supplementary tables S4** and **S5**). The novel variants include 15 missense variants and 3 deletions, their frequencies were evaluated using gnomAD. The pathogenicity of missense variants was evaluated using in silico methods (**Supplementary table S5)**.

The variants identified in this study include 40 substitutions (37 missense, 2 nonsense and 1 splicing variants), 4 deletions, 2 duplications and 1 deletion/insertion. The variant c.245+1G>A is the most prevalent, accounting for 11 % of mutant alleles (11/104). Forty-three variants have been associated to GM1-gangliosidosis phenotype, among them 40 have been identified only in GM1-gangliosidosis patients, while 3 variants (c.442C>T, c.622C>T, c.902C>T) are present in both GM1-gangliosidosis and MPSIVB patients (**figure 2,** (**Supplementary table S5**).

Thirty-one variants have been identified in patients with early-onset GM1-gangliosidosis. Twenty-five variants are present only in early-onset GM1-gangliosidosis including severe variants resulting in a loss of function such as nonsense or frameshift variants (**Supplementary tables S4 and S5**). Twelve variants have been identified only in late-onset GM1-gangliosidosis (**figure 2**).

Seven variants have been linked to MPSIVB and 4 were found only in MPSIVB patients (2T>C, c.323T>C, c.817_818delinsCT, c.1454A>C) (**figure 2**).

DISCUSSION

b-GAL deficiency is characterized by the heterogeneity of the associated clinical phenotypes ranging from neurodegeneration in GM1-gangliosidosis to predominant bone involvement in MPSIVB.

The alteration of both *GLB1* gene products, b-GAL and EBP through the modifications of their production kinetics and homeostasis may contribute to such phenotypic diversity.

More than 200 variants have been reported in *GLB1* gene in the Public Human Gene Mutation DataBase, (HGMD http://www.hgmd.cf.ac.uk/ac/index.php. Accessed June 2020). GM1-gangliosidosis presents an extensive molecular heterogeneity.[1] The most severe form with an early onset before the age of 1 year, is characterized by progressive central nervous system degeneration leading to spasticity, deafness, blindness, and death by 1 and 2 years of age. Storage features are usually present such as dysmorphism, hepatosplenomegaly and skeletal dysplasia. Macular cherry-red spots are less frequently observed. [1 3] A milder clinical phenotype, with a slower progression, is characterized by psychomotor delay, pyramidal and extrapyramidal syndromes, cognitive impairment or regression, and epilepsy. The adult form is rare and the onset may occur as late as the 2nd to 3rd decades with mainly extrapyramidal signs and progressive evolution.[20-22]

In this study, integrative clinical and molecular analyses of 47 GM1-gangliosidosis and 5 MPSIVB patients have been conducted to disentangle the above-mentioned complexity. Forty-seven different variants were identified throughout the sequence of the *GLB1* gene, in all exons except exons 7, 11 and 12 (**figure 3, Supplementary table S5**). Thirty-seven were missense or inframe variants and ten were truncating variants (**figure 2**). The variants are individually infrequent, there is no founder effect or hot spot variants. The most prevalent variant (c.245+1G>A) accounts only for 11% of the mutant alleles (**figure 3, Supplementary tables S4 and S5**). The genotype-phenotype correlation is variable, but some variants are nevertheless associated to specific clinical phenotype subgroups namely early-onset GM1-gangliosidosis, late-onset GM1-gangliosidosis and MPSIVB (**figure 4**). Thus, 43 variants out of 47 are associated to GM1-gangliosidosis phenotype, 7 are identified in MPSIVB patients and only 3 variants are

common to both phenotypes (**figure 3**). In GM1-gangliosidosis group, 31 variants out of 43 have been found in patients with an early-onset, among them 25 were identified only in this subgroup. Only 4 variants are present in both early and late-onset GM1-gangliosidosis (**figure 2**). Some variants such as c.602G>A – p.Arg201His have been described as protective from the severe phenotype and associated with late-onset phenotype GM1-gangliosidosis and MPSIVB[23]. Indeed, this variant has been identified in 5 late-onset GM1-gangliosidosis patients (P3, P21, P31, P34 and P44) at a heterozygous status. In these patients, the second mutated alleles have been previously reported to be associated with a moderate phenotype (patients P3 (c.716C>T – p.(Thr239Met)[7]), P21 (c.75+2dup – p.(?)[9], P34 (c.1038G>C – p.(Lys346Asn)[19] and P44 (c.442C>T – p.(Arg148Cys)[16] respectively. Moreover, in MPSIVB patients, at least one allele carries a moderate effect variant associated only with the MPSIVB phenotype (**Supplementary table S1**).

This study reports 47 variants including 29 known and 18 novel variants. We explored the literature regarding the 29 known variants in order to extract the associated phenotypes. Then, we focused only on phenotypes associated with homozygous state in both literature and our findings to be able to perform a consistent genotype-phenotype correlation. Six variants fulfilled these criteria. Five variants (c.176G>A, c.245+1G>A, c.569G>A, c. 1577dup and c.1733A>G) have been described in early-onset form in both literature and our study. One variant, c.1313G>A, has been associated with late-onset phenotype in our series and it has been reported either in late-onset or MPSIVB in the literature. These consistent findings reinforce the genotype-phenotype correlation in b-GAL deficiency (**figure 5, Supplementary table S6**). On these bases, it may be inferred that patient assignment to a clinical and severity subgroup may benefit from molecular stratification using a variant-driven approach.

This study reports the characterization of 18 novel variants (13 missense or inframe and 5 truncating variants, **figure 2**). For the missense variants, 12 were assumed to be deleterious (class V - pathogenic or class IV - probably pathogenic) based on the prediction of at least two of the following *in silico* algorithms, Align GVGD, SIFT, PolyPhen2 or MutationTaster and M-CAP (**Supplemental table S5**). Hence,

one missense variant, c.1577G>A - p.(Gly526Asp) was classified as benign (class I) and was predicted non-pathogenic by all four algorithms (**Supplemental table S5**). This variant was identified in the homozygous state in two index cases (P7 and P8), who presented with hydrops fetalis. No other genetic alterations were found. The β-galactosidase activity was assessed in cultured amniotic cells and was nearly absent. No splicing effect has been predicted using *in silico* tools. Further studies such as functional minigene-based assays are required to confirm the pathogenic effect of this variant. Besides, the presence of another alteration in the deep intronic sequences is not excluded.

The clinical features are illustrated in **figure 1C** and **1D** according to their frequency and the different phenotypic groups in which they are encountered. All the symptom groups are involved in early and late-onset GM1-gangliosidosis while MPSIVB patients present with only musculoskeletal and dysmorphic features (**figure 1C and 1D**). Regarding the molecular features, at a glance, all the variants involved in GM1-gangliosidosis pathology are linked to the whole clinical spectrum regardless their exonic position. EBP results from the deletion of exons 3, 4 and 6 and exon 5 has a different reading frame. Thus, the EBP and b-GAL sequences are identical to amino acids encoded by exons 1, 2 and 7 to 16 whereas the amino acids encoded by exons 3, 4, 5 and 6 are present only in b-GAL sequences. EBP contains a unique sequence of 32 amino acids corresponding to a specific exon 5 reading frame; this sequence is involved in tropoelastin binding. Thus, variants located in common EBP and b-GAL sequences and variants located exclusively in b-GAL sparing EBP sequence may have the same clinical impact. The function of EBP seems to be altered even when the variant is located in exons not coding for EBP.[7] The undegraded KS may link to EBP, prevents its interaction with elastin complex and thus alters elastogenesis. This is indicative of the multifactorial and complex nature of the processes in which EBP is involved.

Another complexity layer is illustrated by the spatial configuration of these molecules associating two other proteins (protective protein/cathepsin A (PPCA - EC 3.4.16.1) and neuraminidase (EC 3.2.1.18))

with EBP on the cell surface and with β-galactosidase in the lysosome. The stability and function of these proteins are conditioned by the integrity of the multiprotein complexes in which they are embedded.

The coordinated assembly of tropoelastin to constitute elastic fibers can be altered either by impaired EBP or the accumulation of glycosaminolycans. Indeed, impaired elastogenesis has been reported in patients with either GM1-gangliosidosis or MPSIVB [24] but also in patients with MPSI (heparan sulfate and dermatan sulfate accumulation) or Costello syndrome.[25]

The pathophysiological role of altered EBP in the development of GM1-gangliosidosis features is still unclear. Its alteration seems implicated in forms with cardiomyopathy. [24]

CONCLUSION

Clinical and molecular descriptions of b-GAL deficiency have been reported in other populations but in France.[8 14 18 19 23 26-34] In this study, we retrospectively analyzed the clinical presentation and related molecular data of 52 patients with GM1-gangliosidosis and MPSIVB. Forty-seven variants have been characterized and a relative genotype-phenotype correlation has been established with variants contributing specifically to a given phenotypic subgroup.

This study unveils the highest complexity of b-GAL deficiency pathogenesis and expands the community knowledge regarding the molecular and clinical landscape of b-GAL deficiency for a better patient management. The putative genotype-phenotype associations observed in this study emphasize the urgent need for a more integrative multiomic and multimodal studies for a deeper understanding of the biological and functional plasticity of this disease.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Data Availability statement

All data that support the findings are included in the manuscript and in the supplemental data.

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FIGURE LEGENDS

Figure 1. **Series overview.** A) Number of patients in each disease group along with their sex distribution between GM1-gangliosidosis (early or late-onset) and mucopolysaccharidosis type IVB. B) Age distribution. C) Overview of the clinical relationships between GM1-gangliosidosis (early or late-onset) and mucopolysaccharidosis type IVB. The box sizes are proportional to the item frequency. D) Detailed clinical presentation for each clinical phenotype.

Figure 2. **Variant landscape across the early and late-onset GM1-gangliosidosis and mucopolysaccharidosis type IVB**. A Venn diagram showing overlap and disease specific variants are highlighted.

Figure 3. **Visualization of the forty-seven described variants**. The number of variants is indicated inside the circle. C-ter: C-terminal domain; N-ter: N-terminal domain. The circle and variant sizes are proportional to the item frequency.

Figure 4. **Integrative visualization summary of clinical and molecular phenotypes.** Relationships between the clinical features and their underlying molecular alterations including variant, clinical phenotype and symptoms.

Figure 5. **Genotype-phenotype association.** Pathogenic variants in homozygous states and their respective phenotype associations described in both literature and this study.

A

Gangliosidosis GM1 Early onset | Gangliosidosis GM1 Late onset | MPSIVB

Female | Male

20
16
8
3
5

B

Gangliosidosis GM1 Early onset | Gangliosidosis GM1 Late onset | MPSIVB

Female | Male

Frequency

Age at first symptom (Years)

C

Symptom | Disease

Neurological
Musculoskeletal
Gastrointestinal
Ophtalmologic
Dysmorphia
Respiratory
Antenatal
Growth
Cardiac

GM1 Gangliosidosis Early onset
GM1 Gangliosidosis Late onset
MPSIVB

D

MPSIVB | GM1 Gangliosidosis Late onset | GM1 Gangliosidosis Early onset

Symptom		Category
Global developmental delay	23 6	Neurological
Progressive neurologic deterioration	19 8	
Generalized hypotonia	22 4	
Seizures	12 3	
Abnormal pyramidal sign	7 7	
Progressive extrapyramidal movement disorder	7 6	
Limb hypertonia	9 3	
Intellectual disability	4 7	
Gait disturbance	10	
Poor eye contact	7	
Behavioral abnormality	3	
Abnormal bone structure	11 8 2	Musculoskeletal
Kyphosis	9 1	
Skeletal muscle atrophy	4 3 2	
Scoliosis	6 2 1	
Dysostosis multiplex	3 2 3	
Hepatomegaly	16	Gastrointestinal
Dysphagia	13 3	
Splenomegaly	11	
Visual impairment	10 1	Ophtalmologic
Cherry red spot of the macula	6	
Nystagmus	4	
Abnormal facial shape	9 1 4	Dysmorphia
Macroglossia	3	
Abnormality of the respiratory system	10 1	Respiratory
Hydrops fetalis	10	Antenatal
Failure to thrive	8	Growth
Abnormality of the cardiovascular system	5	Cardiac

Number of Patients

c.442C>T p.(Arg148Cys)

GM1 Late Onset — 12
MPS IVB — 4
0
1
3
2
GM1 Early Onset — 25

c.601C>T   p.(Arg201Cys)
c.551A>G   p.(Gln184Arg)
c.602G>A   p.(Arg201His)
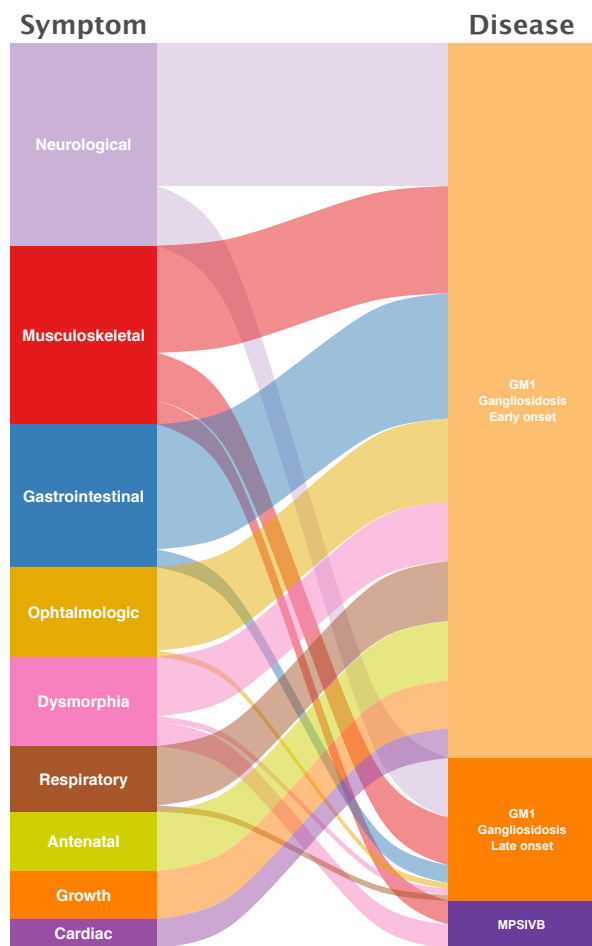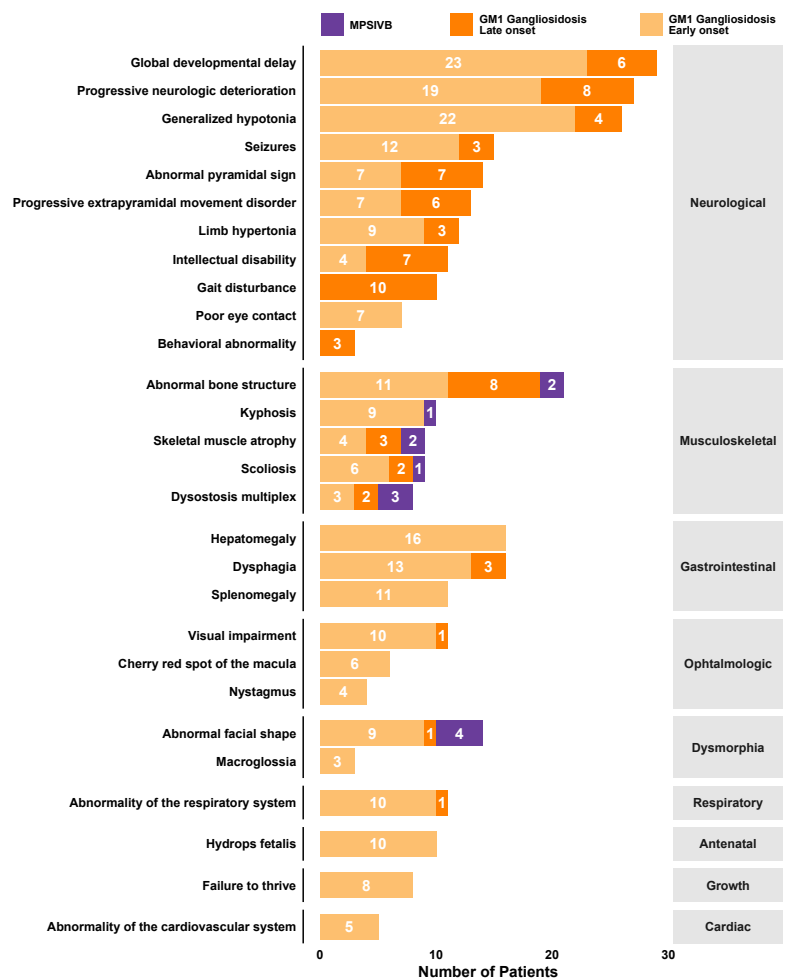c.931G>A   p.(Gly311Arg)
c.1430T>G   p.(Val477Gly)
c.1901G>A   p.(Cys634Tyr)
c.1471G>A   p.(Asp491Asn)
c.1769G>A   p.(Arg590His)
c.1313G>A   p.(Gly438Glu)
c.1038C>C   p.(Lys346Asn)
c.1498A>G   p.(Thr500Ala)
c.216G>A   p.(Met72Ile)

c.1024G>A p.(Asp342Asn)
c.716C>T   p.(Thr239Met)
c.75+2dup p.(?)

c.1454A>C   p.(Tyr485Ser)
c.323T>C   p.(Leu108Pro)
c.817_818delinsCT p.(Trp273Leu)
c.2T>C   p.(?)

c.622C>T   p.(Arg208Cys)
c.902C>T   p.(Ala301Val)

c.439C>T   p.(Leu147Phe)
c.202C>T   p.(Arg68Trp)
c.325C>T   p.(Arg109Trp)
c.1733A>G   p.(Lys578Arg)
c.829T>C   p.(Trp277Arg)
c.149A>G   p.(Tyr50Cys)
c.623G>C   p.(Arg208Pro)
c.443G>A   p.(Arg148His)
c.569G>A   p.(Gly190Asp)
c.176G>A   p.(Arg59His)
c.1370G>A   p.(Arg457Gln)
c.1456G>A   p.(Gly486Ser)
c.808T>C   p.(Tyr270Asp)
c.175C>T   p.(Arg59Cys)
c.1577G>A   p.(Gly526Asp)
c.1810C>T   p.(Pro604Ser)
c.1768C>T   p.(Arg590Cys)
c.856_862del p.(Thr286Glnfs*16)
c.1825_1828del p.(Met609Profs*27)
c.485del   p.(Leu162Trpfs*8)
c.1577dup   p.(Trp527Leufs*5)
c.495_497del p.(Leu166del)
c.1051C>T   p.(Arg351*)
c.1369C>T   p.(Arg457*)
c.245+1G>A   p.(?)

Missense/Inframe 37 variants
Truncating 10 variants

Missense/Inframe 37 variants

Variants shown (from N-ter to C-ter): p.(Tyr50Cys), p.(Arg59Cys), p.(Arg59His), p.(Arg68Trp) 2, p.(Met72Ile), p.(Leu108Pro), p.(Arg109Trp), p.(Leu147Phe), p.(Arg148His), p.(Arg148Cys) 3, p.(Gln184Arg), p.(Gly190Asp), p.(Arg201Cys), p.(Arg201His) 5, p.(Arg208Pro), p.(Arg208Cys) 3, p.(Thr239Met), p.(Tyr270Asp) 2, p.(Trp273Leu) 3, p.(Trp277Arg), p.(Ala301Val) 2, p.(Gly311Arg), p.(Asp342Asn) 2, p.(Lys346Asn), p.(Gly438Glu), p.(Arg457Gln) 2, p.(Val477Gly), p.(Tyr485Ser), p.(Gly486Ser) 3, p.(Asp491Asn), p.(Thr500Ala), p.(Gly526Asp) 2, p.(Lys578Arg) 3, p.(Pro604Ser), p.(Arg590Cys), p.(Arg590His), p.(Cys634Tyr) 2

GLB1
NM_000404
Protein length

N-ter — Catalytic domain — C-ter
50 100 150 200 250 300 350 400 450 500 550 600 650

Exon 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Truncating 10 variants

c.2T>C
c.75+2dup 4
c.245+1G>A 6
p.(Leu162Trpfs*8)
p.(Leu166del)
p.(Thr286Glnfs*16)
p.(Arg351*)
p.(Arg457*)
p.(Trp527Leufs*5) 3
p.(Met609Profs*27)

Figure legend columns — Phenotype (GM1 Early, GM1 Late, MPSIVB) | Genotype (Variant) | Symptoms (Neurological, Musculoskeletal, Gastrointestinal, Dysmorphia, Ophtalmologic, Respiratory, Growth, Antenatal, Cardiac)

Missense / Inframe (Published) (23)

GM1 Early	GM1 Late	MPSIVB	Variant	Neurological (39)	Musculoskeletal (38)	Gastrointestinal (26)	Dysmorphia (21)	Ophtalmologic (20)	Respiratory (15)	Growth (12)	Antenatal (11)	Cardiac (7)
	GM1 Late		c.1038G>C p.(Lys346Asn)	●	●							
	GM1 Late		c.931G>A p.(Gly311Arg)	●	●	●						
	GM1 Late		c.716C>T p.(Thr239Met)	●	●			●			●	
	GM1 Late		c.602G>A p.(Arg201His)	●	●							
	GM1 Late		c.1498A>G p.(Thr500Ala)	●	●	●						
	GM1 Late		c.1313G>A p.(Gly438Glu)	●	●							
	GM1 Late		c.601C>T p.(Arg201Cys)	●	●				●			
	GM1 Late		c.551A>G p.(Gln184Arg)	●	●				●			
GM1 Early			c.443G>A p.(Arg148His)	●	●		●					
GM1 Early			c.1768C>T p.(Arg590Cys)	●	●							●
		MPSIVB	c.817_818delinsCT p.(Trp273Leu)	●	●		●	●				
	GM1 Late		c.1769G>A p.(Arg590His)	●	●		●	●				
	GM1 Late		c.1471G>A p.(Asp491Asn)	●	●	●		●				
GM1 Early		MPSIVB	c.569G>A p.(Gly190Asp)	●	●	●		●				
GM1 Early			c.902C>T p.(Ala301Val)	●	●					●		
GM1 Early		MPSIVB	c.808T>C p.(Tyr270Asp)	●	●					●		
GM1 Early			c.175C>T p.(Arg59Cys)	●	●				●			
GM1 Early			c.622C>T p.(Arg208Cys)	●	●			●				
GM1 Early		MPSIVB	c.442C>T p.(Arg148Cys)	●	●			●				
		MPSIVB	c.176G>A p.(Arg59His)	●	●		●	●	●	●		●
GM1 Early			c.1733A>G p.(Lys578Arg)	●	●		●	●	●	●		
GM1 Early			c.202C>T p.(Arg68Trp)	●	●		●	●	●	●	●	
GM1 Early			c.1370G>A p.(Arg457Gln)	●	●				●	●		

Truncating (Published) (6)

GM1 Early	GM1 Late	MPSIVB	Variant	Neurological	Musculoskeletal	Gastrointestinal	Dysmorphia	Ophtalmologic	Respiratory	Growth	Antenatal	Cardiac
GM1 Early			c.75+2dup p.(?)	●	●	●	●	●	●	●		●
GM1 Early	GM1 Late		c.1369C>T p.(Arg457*)	●	●	●	●	●	●	●	●	●
GM1 Early			c.245+1G>A p.(?)	●	●	●	●	●	●	●		●
GM1 Early			c.1577dup p.(Trp527Leufs*5)	●	●	●		●	●	●	●	●
GM1 Early			c.1051C>T p.(Arg351*)	●	●	●	●	●	●	●	●	
GM1 Early			c.495_497del p.(Leu166del)	●	●							

Missense / Inframe (This study) (13)

GM1 Early	GM1 Late	MPSIVB	Variant	Neurological	Musculoskeletal	Gastrointestinal	Dysmorphia	Ophtalmologic	Respiratory	Growth	Antenatal	Cardiac
	GM1 Late		c.216G>A p.(Met72Ile)	●								
	GM1 Late		c.1430T>G p.(Val477Gly)	●								
	GM1 Late		c.1901G>A p.(Cys634Tyr)	●	●							
			c.1810C>T p.(Pro604Ser)	●				●				
GM1 Early	GM1 Late		c.1024G>A p.(Asp342Asn)	●							●	
GM1 Early	GM1 Late		c.1454A>C p.(Tyr485Ser)	●	●		●					
		MPSIVB	c.439C>T p.(Leu147Phe)	●	●	●		●	●	●		
GM1 Early			c.829T>C p.(Trp277Arg)								●	
GM1 Early			c.623G>C p.(Arg208Pro)								●	
GM1 Early			c.325C>T p.(Arg109Trp)									
GM1 Early			c.1577G>A p.(Gly526Asp)								●	
GM1 Early			c.149A>G p.(Tyr50Cys)	●	●	●	●	●	●	●		
GM1 Early			c.1456G>A p.(Gly486Ser)	●	●		●	●	●	●		

Truncating (This study) (5)

GM1 Early	GM1 Late	MPSIVB	Variant	Neurological	Musculoskeletal	Gastrointestinal	Dysmorphia	Ophtalmologic	Respiratory	Growth	Antenatal	Cardiac
GM1 Early			c.856_862del p.(Thr286Ginfs*16)	●	●	●	●	●		●		
GM1 Early			c.485del p.(Leu162Trpfs*8)	●		●		●		●		
		MPSIVB	c.323T>C p.(Leu108Pro)		●		●					
		MPSIVB	c.2T>C p.(?)								●	
GM1 Early			c.1825_1828del p.(Met609Profs*27)								●	

Phenotype   **Genotype**   **Symptoms**