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Clinical and Molecular Characterization of Mucopolysaccharidosis Type 3A and 3B in a Turkish Series

Bilge Noyan[a, b] Nursel H. Elcioglu[a, c] Abdellah Tebani[d] Soumeya Bekri[d]

[a]Department of Pediatric Genetics, Marmara University Medical School, Istanbul, Turkey; [b]Department of Pediatric Metabolic Diseases and Nutrition, Basaksehir Cam ve Sakura City Hospital, Istanbul, Turkey; [c]Eastern Mediterranean University Medical School, Mersin, Turkey; [d]Department of Metabolic Biochemistry, Normandie University, UNIROUEN, INSERM U1245, CHU Rouen, Rouen, France

Keywords
Mucopolysaccharidosis · Sanfilippo syndrome · Genetics · Lysosomal diseases · Founder effect

Abstract
Introduction: Sanfilippo syndrome or mucopolysaccharidosis type 3 (MPS-3) is a rare condition and its epidemiological data are still not defined. MPS-3 is linked to a deficiency in enzymes involved in heparan sulfate degradation. This biomolecule is neurotoxic and its accumulation underlies the severe central nervous system degeneration observed in this disease. ***Methods:*** Here, we describe 15 Turkish patients with MPS-3A or MPS-3B subtypes. Clinical data upon the diagnosis and during the follow-up as well as molecular characterization are reported. ***Results:*** Two and ten distinct variants were identified in *SGSH* and *NAGLU* gene sequences, respectively. Six variants (*NAGLU* NM_000263.3:c.532–?_c.764+?del, NAGLU NM_000263.3: c.509G>T, *NAGLU* NM_000263.3: c.700C>G, *NAGLU* NM_000263.3:c.507_516 del, *NAGLU* NM dises_000263.3: c.1354 G>A, *NAGLU* NM_000263.3: c.200T>C) have been previously published and 6 are novel (*SGSH* NM_000199.4: c.80T>G, *SGSH* NM_000199.4: c.7_16del, *NAGLU* NM_000263.3: c.224_235del, *NAGLU* NM_000263.3: c.904G>T, *NAGLU* NM_000263.3: c.626C>T, *NAGLU* NM_000263.3: c.1241A>G). *SGSH* NM_000199.4:c.7_16del variation might be caused by a founder effect. ***Conclusion:*** Due to the high rate of consanguinity in Turkey, the incidence of Sanfilippo syndrome might be higher compared to other populations worldwide. Our results contribute to the characterization of rare diseases in Turkey and to improve our knowledge of the clinical, molecular, and epidemiological aspects of MPS-3 disease.

Introduction

Mucopolysaccharidoses (MPSs) are rare hereditary disorders related to impaired catabolism of glycosaminoglycans (GAGs), namely, chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, and hyaluronan [1]. Deficiencies in lysosomal hydrolases involved in GAG metabolism lead to the subsequent lysosomal accumulation of un-degraded GAGs in cells and tissues [1]. According to the accumulated substrate, 7 MPS types are described [1]. Mucopolysaccharidosis type 3 (MPS-3, Sanfilippo syndrome) is one of the most common MPS type with high excretion of urinary heparin sulfate [2]. Four Sanfilippo syndrome subtypes

karger@karger.com
www.karger.com/msy

Correspondence to:
Bilge Noyan, bilgenoyan@yahoo.com

have been identified (MPS-3A, MPS-3B, MPS-3C, and MPS-3D) due to the deficiency of one of the 4 enzymes of heparan sulfate catabolism, respectively, heparan-N-sulfamidase (SGSH-MIM #252900), α-N-acetylglucosaminidase (NAGLU-MIM #252920), acetyl CoA:alpha-glucosaminide N-acetyl transferase (HGSNAT, MIM #252930), and N-acetylglucosamine 6-sulfatase (GNS, MIM #252940) [2]. MPS-3 subtypes are autosomal recessive diseases. Clinical picture is mainly characterized by a severe and progressive degeneration of the central nervous system (CNS) with mild- and late-onset somatic symptoms such as hirsutism, hypertrichosis, hepatosplenomegaly, coarse facial feature, and skeletal abnormalities. Neurodegeneration manifests as intellectual disability, behavioral disturbances, hypoacousia, and speech loss. Death usually occurs at the second or third decade of life; although in some attenuated forms, life expectancy may extend until the fifth and sixth decade [3].

The cumulative incidence of Sanfilippo syndrome is estimated to be 0.28–4.1/100,000 [4–6]. MPS-3A being the most prominent in UK, France, Germany and MPS-3B is being the most prominent in Greece [2]. In Turkey, no incidence analysis has been performed, but it may be higher because of high consanguinity rates. Since MPS-3 basically affects the CNS, therapies such as classical enzyme replacement therapy are not indicated [7]. However, intrathecal enzyme delivery could be an option for MPS-3 in animal models. However, the phase 1-2 study of intrathecal heparan-N-sulfatase therapy in MPS-3A patients does not show the expected neurocognitive outcome [8]. Since only 5–15% enzyme activity is efficient to maintain cellular well-being in lysosomal diseases, gene therapy for MPS-3 is the most promising therapeutic option for recovering the enzyme activity [9, 10]. In this study, we describe the clinical and genetic outcomes, management, and genetic counseling of patients with Sanfilippo syndrome MPS-3A and MPS-3B at the Marmara University Medical Faculty, whom we diagnosed and followed between 2000 and 2020.

MPS-3A results from *SGSH* gene alterations located on chromosome 17q25.3 spanning 11 kb and comprising 8 exons. SGSH precursor containing 502-amino acid glycoprotein is processed to a 482-amino acid glycoprotein. The enzyme is active as a homodimer on heparan sulfates. One hundred forty-five variants have been reported in the *SGSH* gene on the public Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SGSH). Most of them are missense/nonsense variants, small deletions, and insertions. Studies have shown that recombinant protein is endocytosed by cells in culture demonstrating that enzyme replacement therapy may be an option in the treatment of Sanfilippo type A.

MPS-3B disease results from a deficiency in the α-N-acetylglucosaminidase enzyme (symbol: NAGLU). The *NAGLU* gene is located on chromosome17q21 spanning 8.3 kp and comprising 6 exons that encode a 743-amino acid precursor protein.

One hundred sixty-six variants have been reported in the *NAGLU* gene on the public Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=NAGLU). These variants encompass missense, nonsense, insertion, and deletion mutations. The vast majority of *NAGLU* pathogenic variants found in Sanfilippo type B patients are unique to a single individual/family [11].

Materials and Methods

Patients and Sample Collection

Ethical approval for the study was provided by Ethical Committee of the Marmara University Health Institute (date: May 6, 2016, number: 09.2016.318) and all samples from patients were obtained in accordance with the Helsinki Declarations. Written, informed consent was obtained from the legal representatives of the patients.

Six patients with MPS-3A and 9 patients with MPS-3B belonging to 14 distinct families are reported. The diagnosis was based on biochemical exploration (quantification and identification of accumulated GAGs, assay of SGSH, and NAGLU enzyme activity). Molecular study of *SGSH* or *NAGLU* genes has been done for 14 patients. All diagnosed patients with MPS-3A and MPS-3B were evaluated at the outpatient clinic by an experienced pediatrician in every 6 months from the diagnosis between 2002 and 2016. A questionnaire, including the data of past medical history, first clinical signs, developmental milestones, the start of the behavioral problems, date of the functional loss, were filled out by caregivers under the observation of a pediatrician.

Biochemical Diagnosis

Biological diagnosis was oriented by quantitation and electrophoresis of urinary GAGs. The diagnosis was confirmed by the assessment of residual SGSH and NAGLU activity in leukocytes using fluorogenic substrates.

Genetic Analysis

Genomic DNA of the 15 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). *SGSH* and *NAGLU* genes were sequenced using LysoGene panel, which includes 52 lysosomal genes [12]. This next-generation sequencing-based method is implemented on an Illumina® platform (San Diego, CA, USA). As previously described [13], 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 (Sophia Genetics, Switzerland) for variant annotation, and

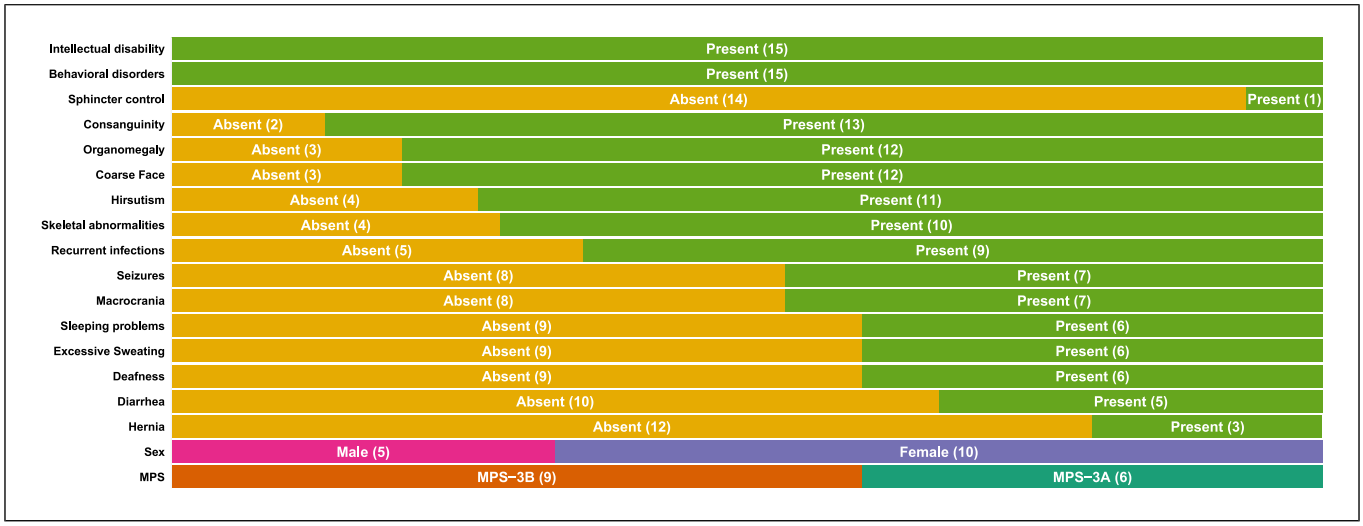

Fig. 1. Overview of the clinical characterization of the series.

Table 1. Overview of medical imaging findings

Patient	Age of imaging, years	Sex	Magnetic resonance imaging
1A	13	Female	Frontal cysts, uncompleted, periventricular, white matter myelinization, ventriculomegaly
3A	6	Female	Ventriculomegaly, increase in the intensity of signaling at posterior, increase in the cortical sulcus, decrease in thickness of corpus callosum
4A	14	Female	Frontal cortical atrophy, uncompleted, periventricular, white matter myelinization, hypodense regions
5A	4	Male	Normal parenchyma, asymmetry of ventricles, hydrocephaly
2B	5	Female	Ventriculomegaly, white matter change, cortical diffuse atrophy, arachnoid cyst, megacysternamagna
4B	16	Male	Normal

CanDiD database for prioritizing and filtering variants of interest. The CANOES algorithm (copy number variants with an arbitrary number of exome samples) allowed the detection of copy number variants such as deletions or duplications. The variants were named according to the nomenclature recommendations (http://www.hgvs.org/mutnomen) using the NM_000199.4 for *SGSH* sequence and NM_000263.3 for *NAGLU* sequence.

Allele Frequency Analysis

The frequency in human population of the novel variants was evaluated using Genome Aggregation Database browser (http://gnomad.broadinstitute.org/; accessed in January 2022).

Computational Analysis of the Functional Impact of Missense Variants

The pathogenicity of missense variants was evaluated using in silico methods including Align GVGD, SIFT, PolyPhen-2, 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.

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/).

Results

Clinical Features

Of our 15 patients, 6 were diagnosed with MPS-3A, 9 of them were MPS-3B. These patients belong to 14 different families, and 13 of the families are consanguineous

(93%). The mean age at diagnosis of the disease is 10.8 years in MPS-3A patients and 6.6 years of age in MPS-3B patients, the youngest age at diagnosis is 1 year (patient 1A), and the oldest age at diagnosis is 16 years (patient 6A). Additionally, there was a prenatal diagnosis and a medical abort in patient 1A's family. As shown in Figure 1, intellectual disability and behavioral disturbances such as hyperactivity and autism spectrum were present in all patients. The other presented clinical signs at their last visit are ranked in the following order of frequency: lack of sphincter control (14/15), coarse face (12/15), organomegaly (12/15), hirsutism (11/15), skeletal abnormalities (10/14), recurrent infections (8/15), seizures (7/15), macrocrania (7/15), sleeping problems (6/15), excessive sweating (6/15), deafness (6/15), diarrhea (5/15), and hernia (3/15) as shown in online supplementary Table S1 (for all online suppl. material, see https://doi.org/10.1159/000535888).

Skeletal involvement was clinically detected as joint contractures. These abnormalities are radiologically identified as mild dysostosis multiplex with cortical thickening of the cranium, costal thickening, and vertebral involvement, irregularities in the metacarpal bones, acetabulum, and femoral head. Multiple joint involvements were detected in our 11 patients (MPS-3A: 5 patients/MPS-3B: 6). Dysostosis multiplex was detected as acetabular head irregularities in 11 of the patients. Other parts of the skeletal involvements were diagnosed, but femoral joint involvement was seen in all the patients having clinical skeletal deterioration at their later ages.

Cranial imaging with MRI of 6 MPS-3 patients had been conducted. Average imaging age of patients who underwent cranial imaging is 9.5. Abnormal cranial imaging was detected in 5 out of 6 MPS-3 patients; most common radiological findings are ventriculomegaly, global atrophy, and delayed myelination in T1 relaxation time and T2 relaxation time as shown in Table 1.

At neuromotor developmental steps' evaluation of the patients, the average age at walking ability onset was 15 months in MPS-3A patients and 14 months in MPS-3B patients. Patients 1A and 1B never speak. The other patients presented with a severe delay in speaking. Among MPS-3A patients, only patient 2A gained the sentence-making ability while 5 out of 8 MPS-3B gained this ability. Regarding the loss of acquired functions at last evaluation, the ability to speak was lost before the age of 10 years in all patients who acquired this ability formerly. Walking ability loss was observed in 5 MPS-3A patients (1A, 2A, 3A, 4A, 6A) in the last evaluation and the average age of the function loss is 10 years of age and they started to use wheelchair. Loss of walking ability was

Table 2. Neurodevelopmental assessment of the series

Patient	1A	2A	3A	4A	5A	6A	1B	2B	3B	4B	5B	6B	7B	8B	9B
Age at diagnosis, years	13	9	6	14	7	16	1	3	9.5	14	12	5	8	5.5	2
Sex	Male	Female	Male	Female	Male	Female	Female	Female	Female	Male	Male	Female	Female	Female	Female
Start of walking, months	14	18	11	24	14	11	14	14	9	12	18	12	12	24	18
Start of the first words, months	Never	24	18	36	12	18	Never	30	18	18	18	15	12	36	18
Start of sentence making, months	Never	24	Never	Never	Never	Never	Never	Never	24	24	24	48	18	Never	Never
Last evaluation, years	13	9	8	18	9	20	6	6	9.5	16	12	7	11	7.5	11
Loss of speaking ability, years	Never speaks	7	9	8	Only words	4	Never speaks	Only words	5		8	6	9	6	9
Unsteady walking, years	4.5	7	8	11		13	2	Unsteady	5			5	6	6	7
Loss of walking ability, years	6	8	9	13		15							10		
Bedridden, years	8	10	10	17		16									
Loss of self-feeding, years	11	6	6	8	5	13.5					8				7
Loss of recognition ability, years	5	6	6	8	3	3.5		1.5	5		8		8		

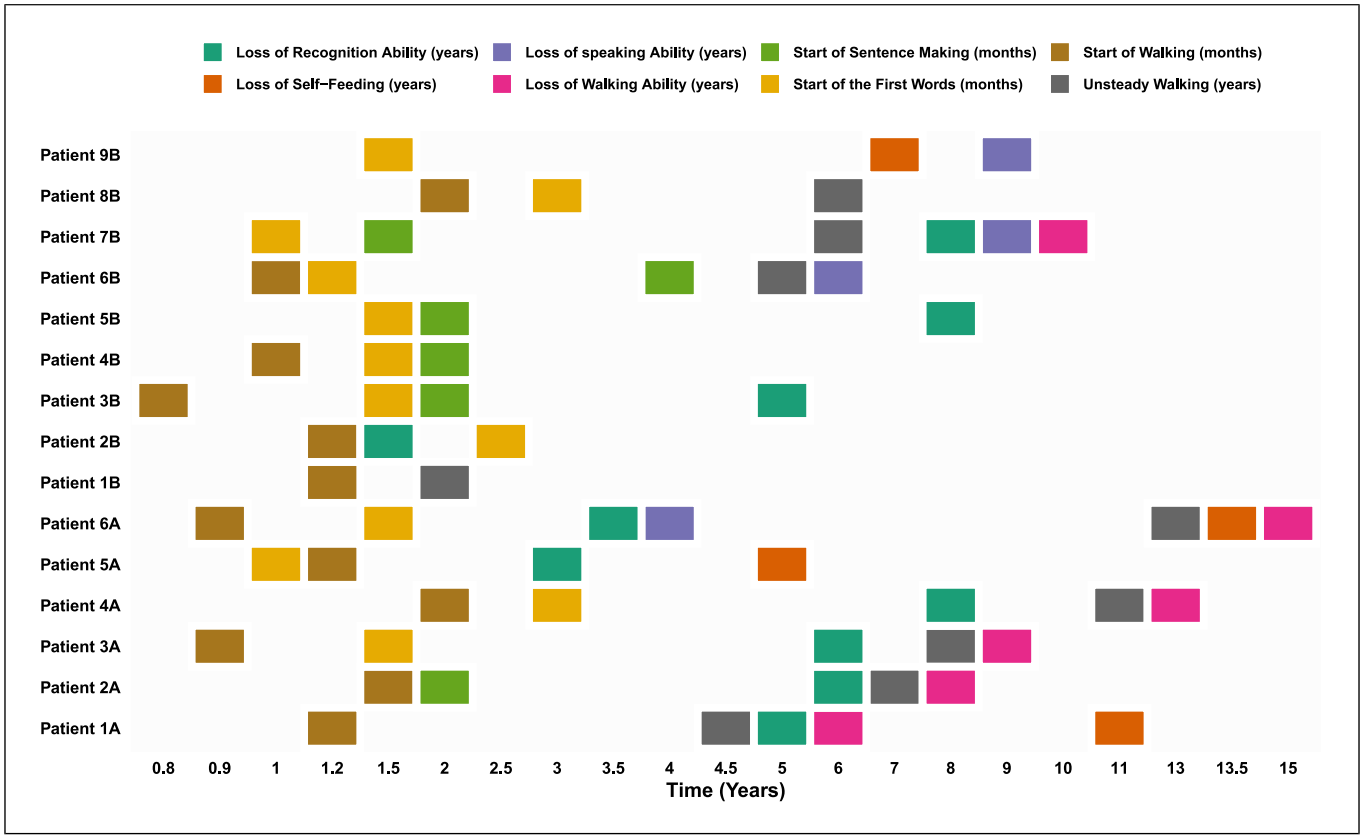

Fig. 2. Developmental evolution of the patients over time.

observed in only 1 MPS-3B patient (7B) at 10 years old. Five of the MPS-3A patients were bedridden and the average age of when they were bedridden is 12 years. None of the MPS-3B patients were bedridden at their last evaluation. Loss of self-feeding was lost in all MPS-3A patients (average age: 8.2 years) and 2 MPS-3B patients (average age: 7.5 years). Loss of recognition of family members was observed in all MPS-3A patients (average age: 5.2 years) and 4 of the MPS-3B patients (average age: 6 years) as shown in Table 2 and Figure 2.

Biochemical and Molecular Characterization

A total of 15 patients with MPS-3A and 3B have been investigated. MPS-3 diagnosis was demonstrated by the elevated urinary HS concentration and the reduction or the absence of SGSH (3A) or NAGLU (3B) enzyme activity in leukocytes in all included patients. Molecular analyses were conducted for 14 patients. Two mutated alleles were identified in all patients and the unaffected parents were heterozygous for one allele. Twelve patients born to consanguineous parents presented with homozygous variants. Two variants have been identified in *SGSH* gene and ten in *NAGLU*. Six variants have been previously published [2, 14–17] and 6 are novel as shown in Table 3. The novel variants include 4 missense variants and 2 deletions; their frequencies were evaluated using Genome Aggregation Database browser. The pathogenicity of missense variants was evaluated using in silico methods. The variants were graded using the ACMG/AMP classification system. The variant SGSH NM_000199.4:c.7_16del is the most prevalent in MPS-3A patients, accounting for 10 out of 12 mutated alleles.

Discussion

The biochemical basis for each of the four types of Sanfilippo syndrome is distinct, but the clinical presentations of MPS-3 types are quite similar. Compared to other types of MPS, MPS-3 syndromes are characterized by limited somatic manifestations and severe CNS involvement and manifest as progressive mental retardation and behavioral problems [18]. Classically, clinical symptoms appear after a free interval of 2–6 years.

Table 3. Molecular findings of the series and their novelty status

Patient	Gene	Exon	Allele	Variant_cDNA	Variant_protein	Novelty
1A	SGSH NM_000199.4	Exon 1	Allele 1	c.80T>G	p.Leu27Arg	Novel
1A	SGSH NM_000199.4	Exon 1	Allele 2	c.80T>G	p.Leu27Arg	Novel
2A	SGSH NM_000199.4	Exon 1	Allele 1	c.7_16del	P.Cys3profs*8	Novel
2A	SGSH NM_000199.4	Exon 1	Allele 2	c.7_16del	P.Cys3profs*8	Novel
3A	SGSH NM_000199.4	Exon 1	Allele 1	c.7_16del	P.Cys3profs*8	Novel
3A	*SGSH* NM_000199.4	Exon 1	Allele 2	c.7_16del	P.Cys3profs*8	Novel
4A	*SGSH* NM_000199.5	Exon 1	Allele 1	c.7_16del	P.Cys3profs*8	Novel
4A	*SGSH* NM_000199.6	Exon 1	Allele 2	c.7_16del	P.Cys3profs*8	Novel
5A	*SGSH* NM_000199.7	Exon 1	Allele 1	c.7_16del	P.Cys3profs*8	Novel
5A	*SGSH* NM_000199.8	Exon 1	Allele 2	c.7_16del	P.Cys3profs*8	Novel
6A	*SGSH* NM_000199.9	Exon 1	Allele 1	c.7_16del	P.Cys3profs*8	Novel
6A	*SGSH* NM_000199.10	Exon 1	Allele 2	c.7_16del	P.Cys3profs*8	Novel
1B	*NAGLU* NM_000263.3	Exon 1	Allele 1	c.224_235del	p.Val75_Arg78del	Novel
1B	*NAGLU* NM_000263.4	Exon 1	Allele 2	c.224_235del	P.Val75_Arg78del	Novel
2B	*NAGLU* NM_000263.5	Exon 3–4	Allele 1	c.532–?_c.764+?del	?	Ozkinay et al. [17] (2021)
2B	*NAGLU* NM_000263.6	Exon 3-4	Allele 2	c.532–?_c.764+?del	?	Ozkinay et al. [17] (2021)
3B	*NAGLU* NM_000263.7	Exon 2	Allele 1	c.509G>T	p.gGy170Val	Ozkinay et al. [17] (2021)
3B	*NAGLU* NM_000263.8	Exon 2	Allele 2	c.509G>T	p.Gly170Val	Ozkinay et al. [17] (2021)
4B	*NAGLU* NM_000263.9	Exon 4	Allele 1	c.700C>G	p.Arg234Gly	Beesley et al. [14] (1998)
4B	*NAGLU* NM_000263.10	Exon 4	Allele 2	c.700C>G	p.Arg234Gly	Beesley et al. [14] (1998)
6B	*NAGLU* NM_000263.11	Exon 2	Allele 1	c.507_516 del	p.ser169Argfs*13	Heron et al. [2] (2011)
6B	*NAGLU* NM_000263.12	Exon 5	Allele 2	c.904G>T	p.glu302*	Novel
7B	*NAGLU* NM_000263.13	Exon 1	Allele 1	c.200T>C	p.Leu67Pro	Kim et al. [16] (2013)
7B	*NAGLU* NM_000263.14	Exon 1	Allele 2	c.200T>C	p.Leu67Pro	Kim et al. [16] (2013)
8B	*NAGLU* NM_000263.15	Exon 3	Allele 1	c.626C>T	p.Thr209Ile	Novel
8B	*NAGLU* NM_000263.16	Exon 6	Allele 2	c.1241A>G	p.His414Arg	Novel
9B	*NAGLU* NM_000263.17	Exon 6	Allele 1	c.1354 G>A	p.Glu452Lys	Bunge et al. [15] (1999)
9B	*NAGLU* NM_000263.18	Exon 6	Allele 2	c.1354 G>A	p.Glu452Lys	Bunge et al. [15] (1999)

Neurological disorders are progressive and prominent, with aggressive behavior, sleep disturbances, hyperactivity, and developmental delay [18]. Severe neurological deterioration begins at the age of 6 years in most patients. Speech delay was the first sign observed in 93% and 88% of patients with MPS-3A and MPS-3B, respectively [2]. In our study, although the behavioral problems are more prominent, somatic features were also broadly present at disease progression (shown in Fig. 1; online suppl. Table S1).

At developmental steps evaluation, early development stages such as head holding, sitting were at normal age compared to their peers. However, late developmental milestones such as walking, speaking, and sentence making seem to be quite backward compared to same-age peers. Therefore, Sanfilippo syndrome should be kept in mind for differential diagnosis of neuromotor developmental delay, especially in speech delay. In our study, 5 MPS-3A patients (85%) and 4 MPS-3B patients (44%) never gained the sentence-making ability. The MPS-3A patient who had sentence-making ability gains this function at 24 months of age; the average age of 5 MPS-3B patients who gained the sentence-making ability was 27 months. Of note, developmental delay <5 years was seen in 96% of MPS-3A patients [19] and 95% of the MPS-3B patients [11].

Sanfilippo type A is the most severe form of the disease exhibiting the earliest onset, most rapid progression of symptoms, and shorter survival [6]. While MPS-3B has the mildest clinical outcome of Sanfilippo syndromes, most reports emphasized clinical variability within disease subtypes, including within multiplex families. Clinical variability of MPS-3 is attributed to high allelic heterogeneity. Large numbers of pathogenic variants have been identified in MPS-3A, MPS-3B, and MPS-3C [14] patients and even with the same variant, clinical outcomes might be different. In our study, the clinical evaluation and time of the loss of functions were better in MPS-3B than in MPS-3A. Molecular study also enabled the identification of the same novel homozygous mutation, *SGSH* NM_000199.4:c.7_16del, in patients 2A, 3A, 4A, 5A, 6A. These 5 patients belong to 4 different families

from the same geographic region of Turkey. Thus, this new variant may be considered as a founder effect. Their clinical features and progression of the disease were similar. Due to the progression of dementia, MPS-3 patients become withdrawn and eventually lose contact with their environment. In our patients, the loss of function was more rapid and severe in MPS-3A patients than in MPS-3B patients.

Conclusions

Thirteen out of 14 families were consanguineous; this high rate may suggest a higher incidence of MPS-3A and 3B in Turkey. Indeed, other Turkish series have been recently reported [17, 19, 20]. Sanfilippo syndrome is a life-threatening disease, and there are no treatment options to date. Therefore, early diagnosis is important for the initiation of supportive therapy. In addition, given the high rate of consanguinity, this early diagnosis may allow genetic counseling to be offered.

Acknowledgment

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Statement of Ethics

Ethical approval for the study was provided by Ethical Committee of the Marmara University Health Institute (date: May 06, 2016, number: 09.2016.318) and all samples from patients were obtained in accordance with the Helsinki Declarations. Written, informed consent was obtained from the legal representatives of the patients.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Authors declare no conflict of interest.

Author Contributions

Bilge Noyan collected the clinical data, performed literature review, and drafted the manuscript including tables. Nursel H. Elçioğlu initiated and supervised this project, conceived the topic of the review, and critically revised and edited the manuscript. Abdellah Tebani performed literature search, analyzed data, and generated figures. Soumeya Bekri conceived the topic and critically revised and edited the manuscript. All authors approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

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