



## Clinical and genetic features of 13 patients with mucopolysaccharidosis type IIIB: Description of two novel *NAGLU* gene mutations

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### ABSTRACT

**Aim:** Mucopolysaccharidosis type III B (MPS IIIB) is an autosomal recessive lysosomal storage disease caused by mutations in the *NAGLU* gene which codes the lysosomal enzyme alpha-N-acetylglucosaminidase. The major symptoms of the disease are cognitive and neurological defects. In this study, the molecular spectrums of 13 MPS IIIB patients were evaluated.

**Material and methods:** Thirteen MPS IIIB patients from 11 families were included in this study. All patients were both clinically and molecularly diagnosed. *NAGLU* gene sequencing was performed using a next generation sequencing platform (Illumina MiSeq). Demographic, clinical and laboratory findings of the patients were obtained via the hospital records.

**Results:** Ten different mutations from the 13 MPS IIIB patients were identified. Eight of the 10 mutations were missense, one was splice site, and one large deletion was also observed. Two mutations c.509G>T (p.Gly170Val) and c.700C>G (p.Arg234Gly) have been defined for the first time in this study.

**Conclusion:** Our study expanded the mutation spectrum of the *NAGLU* gene thereby contributing to the improved genetic counselling of MPS IIIB patients. Confirming the literature, missense mutations were also found to be the most common *NAGLU* mutations in our study.

## 1. Introduction

Mucopolysaccharidosis type IIIB (MPS IIIB), which results from mutations in the  $\alpha$ -N-acetylglucosaminidase (*NAGLU*) gene located at 17q21, is an autosomal recessive lysosomal storage disorder with a prevalence of 1:250000 live births. It is one of the five subtypes of MPS III or Sanfilippo Syndrome. Each of the five subtypes suffers from a different enzyme deficiency: sulfamidase (MPS IIIA),  $\alpha$ -N-acetylglucosaminidase (MPS IIIB),  $\alpha$ -glucosaminide N-acetyltransferase (MPS IIIC), N-acetylglucosamine 6-sulfatase (MPS IIID) and N-glucosamine 3-O-sulfatase (MPS IIIE) [1].

The clinical features of MPS III result from the accumulation of undegraded glycosaminoglycans (GAGs) within the lysosome, and are similar across all subtypes. These include progressive neurodegeneration, hyperactivity, and sleep disorders; as well as somatic

symptoms such as dysostosis and hepatomegaly.

MPS IIIB shows broad spectrum of clinical and genetic heterogeneity. Clinical manifestations range from mild to severe, and are associated with allelic heterogeneity in the *NAGLU* gene.

In this study, we aimed to evaluate the clinical features, molecular analysis results and genotype-phenotype correlation of 13 MPS IIIB patients from 11 families.

## 2. Material and methods

The study included 13 Turkish MPS IIIB patients from 11 different families. The initial diagnosis of MPS IIIB was established based on clinical manifestations. All patients were examined by both an expert metabolic specialist, and a pediatric geneticist. The clinical and biochemical findings of the patients were obtained from their hospital

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records. Written informed consent for genetic testing was obtained in all cases or their parents/guardians. Genomic DNA was isolated from peripheral blood with 2 cc EDTA using the QIAamp DNA Blood Mini Kit (Qiagen Ltd., Crawley, United Kingdom). Appropriate primers were designed to amplify all coding exonic regions and exon-intron junctions of the *NAGLU* gene. Sequence analysis of *NAGLU* gene was performed using a next generation sequencing platform (Illumina MiSeq) with PCR based library preparation. For novel mutations, pathogenicity was classified in accordance with American College of Medical Genetics (ACMG-2015) guidelines.

The study was approved by the Ethical Committee of the Ege University Medical Faculty (Date:16/10/2019, number: 19–10.1 T/28) and all samples from patients were obtained in accordance with the Helsinki Declarations.

### 3. Results

#### 3.1. Clinical findings

Of the 13 MPS IIIB patients nine were female (69,2%). All patients were born to consanguineous parents. The median age for the onset of disease manifestations was 2 years (min:0, max:7), with 4 years (min:1, max:9.5) for the confirmed molecular diagnosis. Twelve patients were referred to our clinic due to developmental and/or speech delay, while one patient was referred due to hepatomegaly.

On dismorphological examination, the most common features observed were coarse face, hepatomegaly and hypertrichosis with the frequencies of 92,3%, 84,6% and 69,2% respectively (Table 1). Neurodevelopmental delays; especially in terms of language ability, were detected in all patients. Enzyme analysis for  $\alpha$ -N-acetylglucosaminidase was performed, and enzyme levels were found to be decreased in all cases. An ophthalmological examination revealed retinal dystrophy and/or optic atrophy in four patients, while hearing loss was detected in five patients. An echocardiogram could only be performed on twelve of the patients, with cardiac anomalies being detected in nine. In one newborn, (P-4) dilated cardiomyopathy was detected. Six patients had been diagnosed with epilepsy. Brain MRIs were available for eleven patients and findings supporting MPS IIIB were observed in all eleven cases. Behavioural problems such as hyperactivity were defined in seven patients and eleven patients had sleeping problems (84,6%). Eight patients were defined to have severe phenotype and five attenuated. The demographic characteristics and phenotypic features of patients were shown in Table 1.

#### 3.2. Molecular results

Ten different mutations in the *NAGLU* gene were identified in 13 patients. Eight of them were missense (%80), one splice site (c.1022-2A>G) (%10), with one (c.532-?\_764+?del) large deletion (%10). Two mutations, c.509G>T (p.Gly170Val) and c.700C>G (p.Arg234Gly), had not been previously reported in the literature. The pathogenicity classification of mutations has been shown in Table 2. Twelve patients carried the same mutation on their both alleles, while one (P-11) was compound heterozygous c.[1000G>A];[1022-2A>G]. Family studies were performed on 7 families, with parents being identified as carrying the variants heterozygously in all cases. The molecular defects of patients are shown in Table 2.

### 4. Discussion

The first symptoms of MPS IIIB typically appear before the age of ten. Patients usually present with a developmental and/or speech delay following an initial symptom-free period. [2]. Confirming the literature, the median age of the disease onset in our study population was also two years (min:0, max:7). In the majority of them, the initial symptom requiring hospital intervention was developmental delay. Previous

studies have shown that there may be changes in the course of disease, with mild course in most patients [3,4]. However, in our study, most patients (61,5%) presented with a severe, wheelchair-dependent picture already requiring full care. The diagnosis of MPS IIIB is based on clinical findings, low enzyme levels and biallelic pathogenic variant determination in the *NAGLU* gene [5]. All our patients met this diagnostic criteria. Clinical findings alone are not sufficient for a diagnosis of MPS IIIB due to the varying spectrum of features across patients. The most common clinical findings are coarse face, hirsutism, hepatosplenomegaly, joint stiffness and hearing loss [5,6]. In our study, coarse face was detected in twelve patients.

Most patients with MPS IIIB have been reported as having hepatosplenomegaly caused by an accumulation of glycosaminoglycans (GAG) and some get diagnosed via organomegaly [6,7]. In our study, eleven patients had hepatomegaly and it was the first identifying feature in patient 3. Joint contractures are associated with severe phenotype and six of our patients presented with contractures.

Progressive degeneration and its consequences vary widely in patients with MPS IIIB. Phenotypic features change from attenuated to severe neurodevelopmental features such as tetraplegia [8,3]. Epilepsy is also not rare additional and can develop with age [7]. It wasn't surprising therefore to find it in six of our patients.

In MPS IIIB patients the most common ophthalmological findings are optic atrophy and retinopathy [7,9]. Both of these findings were observed in our patients (Table 1). Considering cardiovascular anomalies; valvular anomalies specifically are common in MPS IIIB [5]. Six of our 13 patients had valvular anomalies, one VSD and one DCMP.

Behavioural problems including hyperactivity and sleep disorders are commonly seen patients with MPS III [8]. In accordance with previous studies, behavioural problems were observed in the majority of our patients (Table 1).

MPS IIIB shows molecular heterogeneity and, while common mutations have not been reported, three missense mutations relating to codon Arg565 (Arg565Gln, Arg565Trp and Arg565Pro) have been identified in several patients [10–12]. In our series, Arg565Gln was found in one patient (P3) who presented with hepatomegaly and mild intellectual disability.

In genetic disorders, the presence of a genotype-phenotype correlation is useful in terms of genetic counselling. Unfortunately, for many diseases, in the presence of vast variant mutations, it is not possible to accurately predict phenotype [13]. However, a number of pathogenic variants showing genotype-phenotype correlation have been identified in *NAGLU* gene [5]. Homozygosity for nonsense or frameshift pathogenic variants, usually, results in more severe and rapidly progressing phenotypes [14,12]. In our study, neither nonsense nor frameshift mutations were detected. One patient (P-12) did have a large deletion in exon 3–4, and an attenuated phenotype including hearing loss, epilepsy and intellectual disability was observed. Two siblings (P-7–9) had splice site mutation in conjunction with a severe phenotype.

To date, 232 mutations in the *NAGLU* gene have been reported being 169 of them missense [15]. In our study missense mutations were detected in ten patients (76,9%). Missense mutations present with various phenotypes. Certain missense mutations; however, have been found to be associated with severe (p.Val334Phe, p.Pro521Leu) [12,14] or attenuated phenotype (p.Arg643Cys, p.Ser612Gly, p.Glu634Lys and p.Leu497Val) [3]. In our study, one patient (P-11) had compound heterozygous mutations in the *NAGLU* gene c.[1000G>A];[1022-2A>G]. Although the missense variant in codon Val334 has been reported to be associated with severe phenotype, our patient had an attenuated phenotype, that could be explained by a different mutation in the other allele.

Two novel mutations were detected in three different families, with two patients (P4-5 carrying the novel mutation) carrying the same novel mutation (p. Gly170Val) and showing severe phenotype. One of these two patients (Patient 4) was diagnosed as having DCMP during neonatal period. There has been no MPS IIIB patients diagnosed to have DCMP in

**Table 1**  
Clinical findings of MPS IIB patients.

	P-1	P-2	P-3	P-4	P-5	P-6	P-7	P-8	P-9	P-10	P-11	P-12	P-13
Family no	1	2	3	4	5	6	7	8	7	9	10	11	9
Gender	F	F	M	M	F	F	M	F	F	F	M	F	F
Age of onset (years)	2,5	2	7	Neonate	1	2	3,5	2,5	6	1	2	1	1,5
Age at diagnosis (years)	5	7	9,5	1	4	3,5	3,5	5	6	3	2	3	5
Intellectual disability	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatomegaly	+	+	+	+	+	+	+	+	+	+	-	+	-
Coarse face	+	+	-	+	+	+	+	+	+	+	-	+	+
Macrocephaly	-	+	-	-	-	-	+	+	+	+	+	-	+
Hypertrichosis	+	+	-	+	+	+	+	+	+	-	+	N/A	-
Ophthalmologic findings	Optic atrophy	Optic atrophy, retinal dystrophy	N	N	Optic atrophy	N	N	N	N	N	N	Optic disc myelinated by nerve fiber, Exotropia	N
Hearing loss	+	N/A	+	N/A	N/A	+	+	-	-	-	-	+	-
Echocardiography	Bicuspid aortic valve, left ventricular diastolic dysfunction	Myxomatous changes in the mitral and aortic valve	MVP, Hypertrophic intraventricular septum	DCMP, MVP	N/A	Aortic valve insufficiency	N	Mitral valve insufficiency	Aortic stenosis, Minimale mitral valve insufficiency	MVP	N	VSD, pulmonary stenosis,	N
Cranial MRI	Thinning of the corpus callosum, colpocephalic enlargement in the lateral ventricles	Cerebral cortical atrophy	Hyperintense areas in white matter	Corpus callosum hypoplasia, white matter hypomyelination	Cerebral cortical atrophy	Mild cerebral atrophy, hydrocephalus	Hypointense areas in the periventricular and subcortical white matter of the occipital lobes in both cerebral hemispheres, and gliosis areas	N/A	N/A	Abnormalities in white matter, ventriculomegaly	Enlarged subarachnoid and perivascular spaces diffuse cerebral atrophy	Diffuse cerebral atrophy	Diffuse cerebral atrophy, ventriculomegaly
Epilepsy	+	-	-	+	+	+	-	-	-	+	-	+	-
Hyperactivity	+	+	-	+	-	-	-	+	-	+	+	-	+
Sleep disorder	+	+	N/A	+	+	+	-	+	+	+	+	+	+

F: Female, M: Male, N/A: Not applicable, N: Normal, MVP: Mitral valve prolapsus, DCMP: Dilated cardiomyopathy, VSD: Ventricular septal defect,

**Table 2**  
Molecular findings of MPS IIIB patients.

	Genotype	Protein	Location	Novelty	ACMG
P-1	c.[2045T>G];[2045T>G]	Leu682Arg	Exon 6	Known	LP
P-2	c.[230T>G];[230T>G]	Val77Gly	Exon 1	Known	LP
P-3	c.[1694G>A];[1694G>A]	Arg565Gln	Exon 6	Known	P
P-4	c.[509G>T];[509G>T]	Gly170Val	Exon 2	Novel	VUS
P-5	c.[509G>T];[509G>T]	Gly170Val	Exon 2	Novel	VUS
P-6	c.[235G>T];[235G>T]	Gly79Cys	Exon 1	Known	LP
P-7	c.[1022-2A>G];[1022-2A>G]		Intron 5	Known	P
P-8	c.[700C>G];[700C>G]	Arg234Gly	Exon 4	Novel	LP
P-9	c.[1022-2A>G];[1022-2A>G]		Intron 5	Known	P
P-10	c.[934G>A];[934G>A]	Asp312Asn	Exon 5	Known	LP
P-11	c.[1000G>A];[1022-2A>G]	Val334Ile	Exon 5/ Intron 5	Known/ known	VUS/ P
P-12	c.[532-?_764+?del]; [532-?_764+?del]		Exon 3-4	Known	
P-13	c.[934G>A]; [934G>A]	Asp312Asn	Exon 5	Known	LP

LP: Likely pathogenic.

P: Pathogenic.

VUS: variant of uncertain significance.

such an early period. This has been considered as specific to this mutation however further confirming cases are necessary due to the other patient (P-5) had no cardiovascular findings to date. He began experiencing seizures prior to one year old. He had a severe neurodevelopmental delay with dysostosis multiplex, hepatomegaly, corpus callosum hypoplasia and white matter anomalies on cranial MRI. Patient 5 was referred to our clinic with developmental delay at one year old. Epilepsy, hepatomegaly, dysostosis multiplex, joint contractures had all developed before her follow-up examination. She also had cerebellar and optic atrophy. However, while both patient 4 and patient 5 were considered as having severe phenotype, macrocephaly was not detected. They also showed some significant differences. Patient 4 had DCMP, while patient 5 had no cardiac problem to date; and in addition to both patients having dysostosis multiplex, only patient 5 had joint contracture.

The other novel mutation was p.Arg234Gly (P-8) and the patient having this mutation presented with an attenuated phenotype. The patient was referred to our clinic at the age of 2,5 years with neurodevelopmental delay and autism spectrum disorder. She had no ophthalmological features, hearing loss or epilepsy. Her speech skills were delayed but it was less severe than that seen in severe phenotype. She also had dysostosis multiplex, but without joint contractures.

In this study, we also had the opportunity to compare the clinical features of siblings (Family-7 and Family-9). While they showed a similar progression and phenotype, not all their clinical findings were exactly the same. In the family-7 (P-7 and P-9) both siblings had a severe phenotype which included intellectual disability, coarse face, hepatomegaly, dysostosis multiplex, macrocephaly, hirsutism, joint contracture at the elbow. Epilepsy, behavioural abnormality and ophthalmological findings were not observed. However, several different clinical findings were observed. Patient-9 had cardiac abnormalities and sleep disorder, while patient -7 had hearing loss.

In family-9 both patients (P-10 and P-13) presented an attenuated phenotype. They both had intellectual disability, coarse face, ventriculomegaly, behavioural abnormality, sleep disorder and macrocephaly. Ophthalmological findings, hearing loss, hirsutism and joint contractures were not observed. Additionally, Patient 10 had epilepsy and mitral valve prolapsus (MVP).

In conclusion, our study has expanded the mutation spectrum of the *NAGLU* gene, contributing to the improved genetic counselling of MPSIIIB patients. Confirming the literature, missense mutations were also the most common mutations identified in our study. Since few patients (especially siblings) carrying the same mutations showed similar phenotypic features. It has been considered that there may be phenotype-genotype correlation for a number of *NAGLU* mutations. However further studies including more patients are needed to achieve certain decision.

## Statement of ethics

Samples from the patients were obtained in accordance with the Helsinki Declarations. Written informed consent for genetic testing was obtained from all patients and/or their parents/guardians.

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None.

## Author contributions

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## Declaration of Competing Interest

All authors declare that they have no conflict of interest.

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