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Identification and characterization of six new mutations in *GLB1* gene in Ukrainian patients with GM1 gangliosidosis and Morquio B disease

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GM1-gangliosidosis (MIM# 230500) and mucopolysaccharidosis IVB (Morquio B, MIM# 230500) are autosomal-recessive diseases, which belong to the group of lysosomal storage disorders and are caused by changes in the structure of the same gene, *GLB1*. The mutations in *GLB1* lead to deficiency in acid β-galactosidase, and, as a result, the accumulation of GM1-gangliosidosis and keratan sulphate in the patients' lysosomes. **Aim.** To analyze the spectrum of mutations in *GLB1* in Ukrainian patients with GM1 gangliosidosis and Morquio B disease. **Results.** We analyzed *GLB1* in 25 Ukrainian patients with the diagnosis of GM1-gangliosidosis and one patient with Morquio B disease; 52 alleles were analyzed. Seventeen types of pathogenic mutations were identified, including 11 missense replacements, three deletions, one insertion and two mutations in the splicing site. The missense mutation p.His281Tyr (c.841C>T) in exon 8 was found to be the most common, as it was found in 19 out of 52 mutant alleles (36.5 %) of all the examined patients. The study allowed to identify six new mutations, which had not been found in any databases or previously described in scientific literature, including three deletions (c.699delG, c.833delG, c.1203_1205delTTA), two missense replacements (p. Gly243Arg, p.Gly262Ala) and one mutation in the splicing site (IVS12+8T>C). **Conclusions.** Our results can be used for a precise molecular diagnostics of patients with GM1-gangliosidosis and Morquio B disease and prenatal diagnostics in the high risk families.

Keywords: GM1-gangliosidosis, Morquio B disease, gene *GLB1*.

Introduction

GM1-gangliosidosis (MIM# 230500) and mucopolysaccharidosis IVB (Morquio B, MIM# 230500) are autosomal-recessive diseases, which belong to the group of lysosomal storage disorders and are caused by changes in the structure of the same gene – *GLB1* [1]. The mutations in gene *GLB1* lead to the deficiency in acid β-galactosidase, and, as a result, the accumulation of GM1-gangliosidosis and keratan sulphate in the patients' lysosomes.

GM1-gangliosidosis is a clinically heterogeneous, neurodegenerative disease. The general incidence rate of this disease in the world is 1:100,000–1:200,000 newborns [2, 3]. GM1-gangliosidosis may be divided into three clinical forms: type I (infantile form), type II (juvenile form), type III (adult form). In patients with the infantile form, the disease is manifested during their first six months of life with severe pathology of the central nervous system. The juvenile form is characterized by neurological changes and disorders in the locomotor system, which are manifested at the age from 7 months to 3 years. The adult form of the disease is manifested at the age from 3 to 30 years and is characterized by the disorders in the coordination of movements, dysto-

nia, developmental language disorders, short stature and moderate changes in the skeleton.

Morquio B disease is referred to mucopolysaccharidosis type IV and is remarkable for multiple skeletal changes, short stature, corneal opacity, and disorder of the cardiac function [3]. The incidence of Morquio B disease in the world has a wide range from 1:75,000 newborns in the Northern Ireland [4] to 1:640,000 newborns in Western Australia [5]. In contrast to GM1-gangliosidosis, the patients with Morquio B disease do not have neurological symptoms. It is possible that the mutations, responsible for the deficiency in β-galactosidase, have different impact on the ability of the enzyme to split different substrates (GM1-ganglioside and keratan sulphate). However, recently the borders between GM1-gangliosidosis and Morquio B disease have become less and less distinct, as some identified mutations in the gene *GLB1* caused the intermediate phenotype with neurological symptoms and skeletal changes in the patients [1].

The gene *GLB1* is mapped on chromosome 3 in the locus 3p21.33, is 62.5 kb long, consists of 16 exons and encodes 677 aminoacid residues of acid β-galactosidase, including 23 aminoacids of the signaling peptide [6]. The molecule of human acid β-galactosidase is a protein, consisting of three domains (the main catalytic TIM barell domain and β1- and β2- domain), which exist in the monomer form at neutral pH [2]. In conditions of acid pH of the lysosome (pH 4–5) the molecule of human β-galactosidase acquires its active form by dimerization. At present 144 mutations in the gene *GLB1* have been described, 109 of which are missence/nonsense mutations, 14 – deletions, 10 – insertions, 11 – mutations, causing splicing disorders [6].

Noteworthy, the differential diagnostics of GM1-gangliosidosis and Morquio B disease is complicated to the common metabolic deficiency, so the final determination of the diagnosis in the patients with the deficient activity of β-galactosidase requires the molecular and genetic diagnostic methods. The identification of the primary genetic deficiency has become very relevant recently, when there are active investigations on a possible gene correction and an enzyme-replacement therapy for the patients with GM1-gangliosidosis and Morquio B disease. Also it is impossible to have prenatal diagnostics in the high risk families without the identification of the mutation, which triggered the disease.

Until today, there has been no analysis of the mutations in the gene *GLB1* for Ukrainian patients with GM1-gangliosidosis and Morquio B disease and their frequencies are unknown.

The aim of our work was to analyze the spectrum of mutations in the gene *GLB1* in Ukrainian patients with GM1 gangliosidosis and Morquio B disease.

Materials and Methods

The analysis of mutations in the gene *GLB1* was carried out in 26 patients from different regions of Ukraine in the Clinical Genetics Laboratory, NCSH OKHMATDYT, who had the preliminary diagnosis of GM1-gangliosidosis (25 patients) and Morquio B disease (1 patient), determined by the identification of the activity of acid β-galactosidase enzyme and the level of excretion for oligosaccharides and keratan sulphate with urine [7, 8]. Genomic DNA was isolated from peripheral blood of patients using commercial kits NeoSorb (Neogene, Ukraine) according to [the] manufacturer's instructions. All 16 exons of the gene *GLB1,* including the 5′ and 3′ untranslated regions and intron/exon boundaries, were amplified from genomic DNA using thermal cycler SimpliAmp (AB, USA). PCR primers were designed using the Primer 3 program, web-version 4.0.0 (http://bioinfo.ut.ee/primer3/) (Tab. 1).

The identification of mutations was made by the Sanger's method of direct automated sequencing using ABI Prism 3130 device (Applied Biosystems) according to the manufacturer's protocol. The analysis of sequencing results was made using programs Chromas and BLAST (http://www.ncbi.nlm.nih.gov/blast). The electronic databases of known mutations – dbSNP, 1000 Genomes, HGMD – were used to characterize the mutations. The analysis of the pathogenicity of new mutations was made using programs PolyPhen2 and Provean (http://genet-

Table 1. **Primers for sequence in the *GLB1* gene.**

Exon	Primers	Sequence	Tm, °C	The size PCR, bp
1	F	cagggaagacgcctgcaaaa	65.7	473
	R	ggtccactgcctgcgttcc	66.5	
2	F	acctggcttagcaatggttt	58.7	468
	R	ccctcccagaacatcacact	59.9	
3	F	aaagcgccttctccctctt	60.5	398
	R	acctgtgctgggtacagtcc	60.0	
4	F	aaagcccagctcaaataccc	60.5	320
	R	gtctcgaactcccaacctca	60.2	
5	F	acccagccagaaaactcaga	59.8	392
	R	atgccttcccaaatgcaa	60.0	
6	F	gcagtcattaagccttgtcatctta	60.9	398
	R	tcaatctgcccatgacactta	59.1	
7	F	cttgggtgttaagttccaaca	57.6	340
	R	tgactccacaatcccattagc	59.9	
8-9	F	ggcattgctggtattggaag	60.5	598
	R	agcctgggtgacagagtgag	60.5	
10	F	agggtcacgtctgtgtctcc	60.2	389
	R	tagctgatgcctcccttcat	59.8	
11	F	ccagatcctgctttggaaga	60.3	381
	R	cccattaggcttgcagaaaa	60.2	
12	F	ctgggaagcaatactgagtgg	59.7	368
	R	acctgggatctgatgcattt	59.4	
13	F	cgggaggtggaggaagatt	61.4	369
	R	aaagatgatgggtagagcctga	60.1	
14	F	aagcatttctttccaagtctgg	59.8	398
	R	aactcctggcctcaagtgat	58.8	
15	F	cgaggttcatttcctgttgg	60.5	499
	R	aacgccacactcaaaacca	60.1	
16	F	gcatttctttcctccattgc	59.6	810
	R	tggtttattcagcccactca	59.1	

ics.bwh.harvard.edu/pph2/, http://provean.jcvi.org/index.php).

The blood samples of 50 voluntary donors (control group) were analyzed to confirm the pathogenicity of new mutations. The voluntary donors aged 18 to 60 years without any clinical signs of lysosomal pathology gave written consent to the study. To exclude a polymorphic character of new rearrangements the RFLP analysis was used: for c.699delG – PstI digestion, for p.Gly262Ala – Sau96I digestion, for c.833delG – BmiI digestion, for c.1203_1205delTTA; c.1207C > A – PseI digestion.

The parents and patients provided written consent to conduct molecular studies of blood samples of children. Committee on Bioethics allowed the study.

Table 2. **The mutations in gene *GLB1* in Ukrainian patients**

Exons	Nucleotide changes	Aminoacid changes	Share of alleles	Type of mutation	Information sources
2	c.176G > A	p.Arg59His	3/52	missence	Silva C. *et al*, 1999 [9]
intron 3	c.397-2A > G	IVS4 -2A > G	1/52	splicing site	Bidchol A. M. *et al*, 2015 [6]
5	c.481T > G	p.Trp161Gly	3/52	missence	Caciotti A. *et al*, 2011 [3]
6	c.602G > A	p.Arg201His	1/52	missence	Oshima A. *et al,* 1991 [10]
6	**c.699delG**	**p.Leu233fs**	6/52	deletion	**Our studies**
6	**c.727G > A**	**p.Gly243Arg**	1/52	missence	**Our studies**
7	c.765G > C	p.Gln255His	5/52	missence	Iwasaki H. *et al*, 2006 [11]
7	**c.785G > C**	**p.Gly262Ala**	1/52	missence	**Our studies**
8	c.808T > G	p.Tyr270Asp	1/52	missence	Paschke E. *et al*, 2001 [12]
8	**c.833delG**	**p.Gly278fs**	1/52	deletion	**Our studies**
8	c.841C > T	p.His281Tyr	19/52	missence	Paschke E. *et al*, 2001 [12]
8	c.817_818 TG > CT	p.Trp273Leu	1/52	missence	Oshima A. *et al*, 1991 [10]
12	**p.Tyr402del; p.Pro403Tre**	**c.1203_1205delTTA; c.1207C > A**	2/52	deletion; missence	**Our studies**
intron12	**c.1233+8T > C**	**IVS12+8T > C**	1/52	splicing site	**Our studies**
14	c.1445G > A	p.Arg482His	1/52	missence	Oshima A. *et al*, 1991 [10]
15	p.Glu534fs	c.1601_1602 insGCCA	3/52	insertion	Hofer D. *et al*, 2009 [13]
16	c.1768C > T	p.Arg590Cys	1/52	missence	Boustany R. M. *et al*, 1993 [14]

Results and Discussion

While conducting the molecular analysis of the gene *GLB1* in 25 Ukrainian patients with the diagnosis of GM1-gangliosidosis and 1 patient with Morquio B disease, 52 alleles were analyzed. 17 types of pathogenic mutations were identified, including 11 missence replacements, 3 deletions, 1 insertion and 2 mutations in the splicing site (Table 2).

While analyzing the frequencies of the mutations, identified by us during the molecular analysis of Ukrainian patients with GM1-gangliosidosis, it should be noted that the missence mutation p. His281Tyr (c.841C > T) in exon 8 was the most common one. It was found in 19 out of 52 mutant alleles (36.5 %) of all the examined patients and caused the development of the infantile form of the disease. This mutation was present in 4 patients in the homozygous state and 11 patients – in the heterozygous state. The analysis of the data, published in recent 10 years regarding the distribution of the mutation p.His281Tyr in other populations, was conducted by us (Table 3).

The result of the analysis demonstrated that the mutation p.His281Tyr (c.841C > T) was described for single cases of patients from Germany, Italy, and Portugal, whereas no allele with this replacement was identified in other countries. This fact may testify to a considerably higher frequency of this mutation in Ukraine, compared to most countries and allows considering this mutation to be major for patients, suffering from GM1-gangliosidosis in Ukraine. The rest of mutations were observed with a smaller frequency or in single cases.

All the mutations, the prevailing majority of which, previously described in the scientific literature (Table 2), had pathogenic impact on the protein and caused the development of GM1-gangliosidosis or Morquio B disease. Most mutations (12 out of 17) were localized in exons, corresponding to TIM barell of the protein structure domain of β-galactosidase,

Table 3. **Distribution of mutations p.His281Tyr (c.841C>T) in different populations.**

Country	The number of alleles with mutation p.His281Tyr of the general study of alleles	Frequency, %	References
Ukraine	**19/52**	**37**	**This work**
Argentina	0/38	0	Santamaria R. *et al.*, 2007 [15]
Brazil	0/130	0	Fernanda Sperb *et al.*, 2013 [16]
Portugal	2/28	7	Coutinho MF *et al.*, 2012 [17]
Austria	0/32	0	Hofer D. *et al.*, 2010 [18]
Germany	1/34	3	Paschke E. *et al.*, 2001 [12]
Spain	0/14	0	Santamaria R. *et al.*, 2007 [19]
Italy	2/50	4	Caciotti *et al.*, 2011 [3]
UAE	0/28	0	Fatma A. *et al.*, 2013 [20]
Turkey	0/10	0	Başak Çeltikçi *et al.*, 2012 [21]
India	0/100	0	Bidchol, A.M., *et al.*, 2015 [6]
China	0/10	0	Yang *et al.*, 2010 [22], Hong-Lin Lei *et al.*, 2012 [23]

3 more mutations – to β1-domain and 2 mutations – to β2-domain of the protein (Fig. 1).

The electronic databases of known mutations – dbSNP, 1000 Genomes, HGMD – and the published data were used to characterize the mutations and their impact on the formation of the disease phenotype. The mutations, described in the scientific literature, which promoted the formation of the early form of the disease, were found in the genotype of most patients with the infantile form of GM1-gangliosidosis (Table 4).

Two mutations in two different alleles were identified in one patient with the juvenile form of GM1-gangliosidosis (Table 4), one mutation is associated with the onset of the juvenile form of the disease (p.Arg201His), and another one (p.His281Tyr) is re-

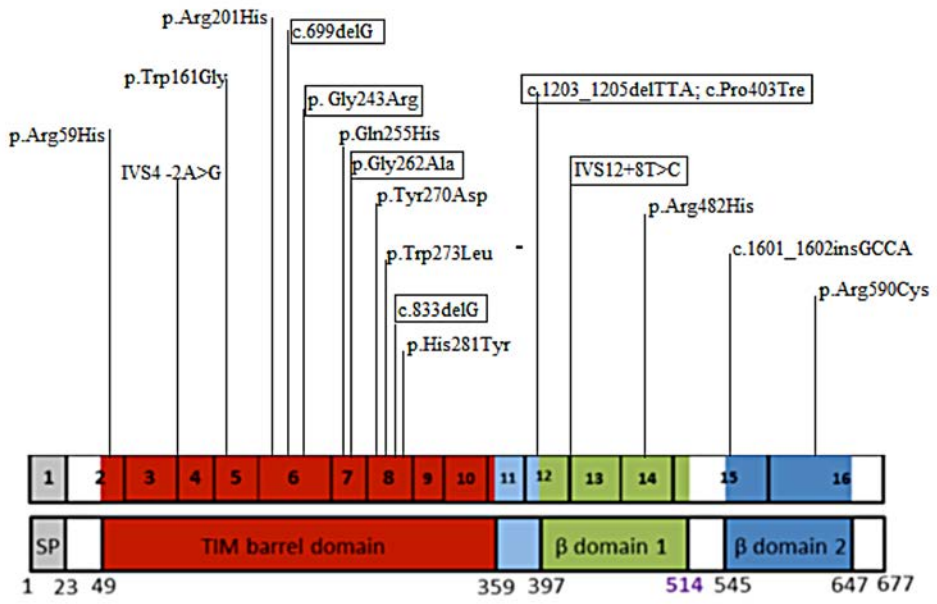

Fig. 1. The localization of known and new (inside the frame) mutations, identified during our study, in exons of the gene *GLB1*, and the scheme of the domain structure of β-galactosidase protein

Table 4. **The genotype and phenotype of 26 Ukrainian patients with GM1-gangliosidosis and Morquio B disease**

Patients	Age at the moment of the visit	Phenotype	First allele	Second allele
1	12 months	Infantile form of GM1	**p.Gly243Arg**	p.His281Tyr
2	8 months	Infantile form of GM1	p.Gln255His	p.His281Tyr
3	9 months	Infantile form of GM1	p.Gln255His	p.His281Tyr
4	10 months	Infantile form of GM1	**c.699delG**	**c.833delG**
5	3 months	Infantile form of GM1	IVS4 -2A>G	p.His281Tyr
6	12 months	Infantile form of GM1	c.1601_1602insGCCA	p.His281Tyr
7	12 months	Infantile form of GM1	**c.699delG**	p.Arg59His
8	13 months	Infantile form of GM1	p.His281Tyr	p.His281Tyr
9	8 months	Infantile form of GM1	**c.699delG**	p.Tyr270Asp
10	14 months	Infantile form of GM1	p.Gln255His	p.His281Tyr
11	12 months	Infantile form of GM1	p.Trp161Gly	p.His281Tyr
12	14 months	Infantile form of GM1	p.Arg59His	p.His281Tyr
13	1 month	Infantile form of GM1	**c.699delG**	p.Arg59His
14	12 months	Infantile form of GM1	p.His281Tyr	p.His281Tyr
15	10 months	Infantile form of GM1	c.1601_1602 insGCCA	c.1601_1602 insGCCA
16	4 months	Infantile form of GM1	p.Trp161Gly	p.Trp161Gly
17	9 months	Infantile form of GM1	p.His281Tyr	p.His281Tyr
18	12 months	Infantile form of GM1	**c.1203_1205delTTA; c.1207C>A**	**c.1203_1205delTTA; c.1207C>A**
19	14 months	Infantile form of GM1	p.His281Tyr	p.His281Tyr
20	4 months	Infantile form of GM1	**c.699delG**	**c.699delG**
21	7 months	Infantile form of GM1	**p.Gly262Ala**	p.Arg590Cys
22	9 months	Infantile form of GM1	p.Trp161Gly	p.Arg482His
23	11 months	Infantile form of GM1	p.Gln255His	p.His281Tyr
24	4 months	Infantile form GM1	p.Gln255His	p.His281Tyr
25	11 y.o.	Juvenile form of GM1	p.Arg201His	p.His281Tyr
26	7 y.o.	Morquio B disease	p.Trp273Leu	**IVS12+8T>C**

the mutations, not described in the scientific literature but identified during our study, are in bold.

markable for the onset of the infantile form of GM1-gangliosidosis and was found to be major for our patients. In this case the mutation p.Arg201His, which affects the development of a milder form of the disease than the mutation p.His281Tyr, had a dominating impact on the development of the patient's phenotype, which confirms the data of other authors [1].

A new mutation IVS12+8T>C and a known mutation p.Trp273Leu, which, according to the scientific literature, is remarkable for this syndrome and must have had the main impact on the formation of the patient's phenotype [13], were found in one patient, whose diagnosis was determined to be Morquio B using the combination of clinical signs and the results of biochemical tests. The mutation p.Trp273Leu has been never observed in the group of patients with GM1-gangliosidosis, examined by us, which testifies to its specific association with Morquio B disease and allows using the results of the molecular analysis for the purpose of differential diagnostics of GM1-gangliosidosis and Morquio B disease.

Six out of 17 mutations in the gene *GLB1* had not been found in any electronic database (dbSNP, 1000 Genomes, HGMD) or previously described in scientific literature, including 3 deletions (c.699delG, c.833delG, c.1203_1205delTTA), 2 missence replacements (p.Gly243Arg, p.Gly262Ala) and 1 nucleotide replacement in the splicing site (IVS12+8T>C) (Fig. 2). Also, none of the identified new mutations was found in the samples of 50 volunteers donors.

The prognostic analysis of possible impact of new mutations on the structure and function of the protein of β-galactosidase was carried out using programs MutationTaster, PolyPhen2 and Provean. The results of this study demonstrated that all new mutations were pathogenic and led to the changes in the structure and functional properties of the protein of β-galactosidase.

The newly found deletions c.699delG in exon 6 and c.833delG in exon 8 lead to the early onset of a

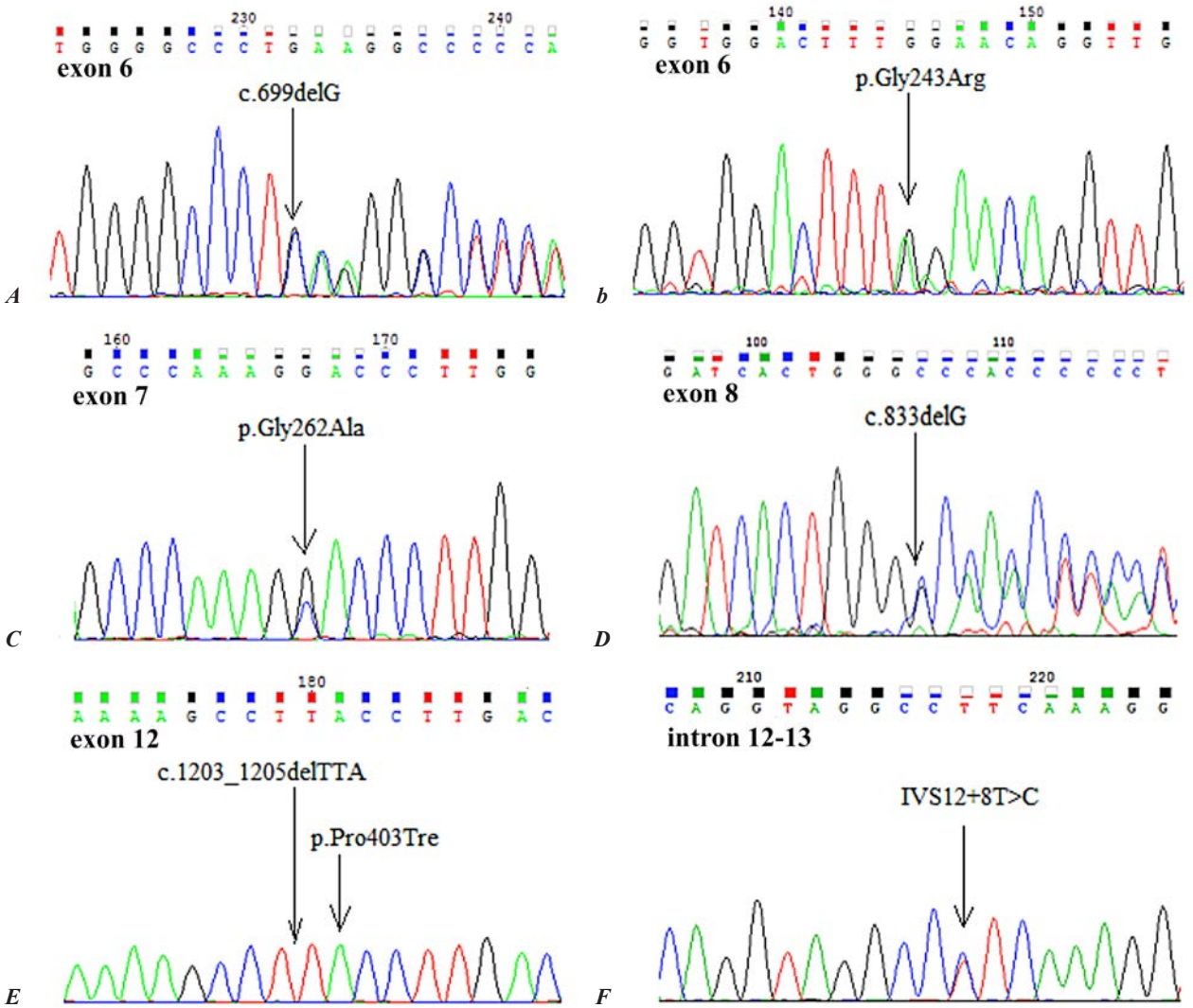

Fig. 2. DNA sequences chromatogram of new mutations in the gene GLB1: *A* – c.699delG in the heterozygous state in exon 6; *B* – p.Gly243Arg in the heterozygous state in exon 6; *C* – p.Gly262Ala in the heterozygous state enkzoni 7; *D* – c.833delG in the heterozygous state in exon 8; *E* – c.1203_1205delTTA combined with p.Pro403Tre mutation in the homozygous state in exon 12; *F* – splice site mutation IVS12 + 8T> C in heterozygous state in 12-13 introns.

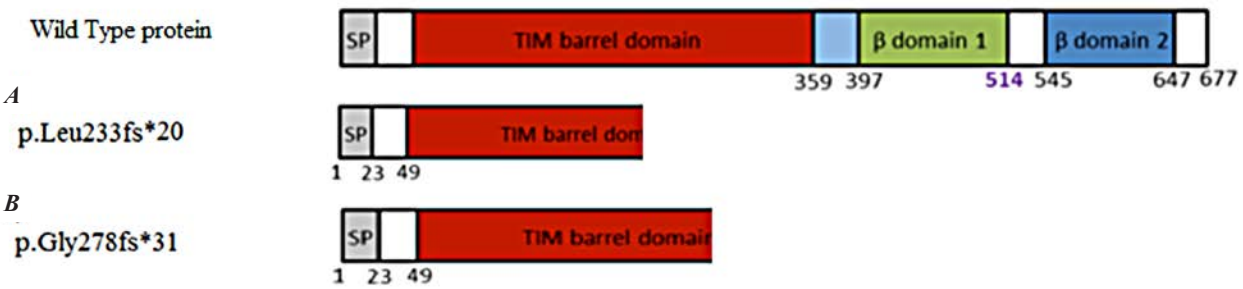

Fig. 3. The schematic presentation of the shortened protein of β-galactosidase due to deletions c.699delG (p.Leu233fs) – *A* and c.833delG (p.Gly278fs) – *B*, which lead to premature occurrence of a stop-codon

stop-codon via 20 aminoacids and via 31 aminoacids respectively. Due to these changes the protein molecule is shorter for deletion c.699delG – by 424 aminoacids, and for c.833delG – by 368 aminoacids, which makes its functioning impossible (Fig. 3).

The deletion c.1203_1205delTTA, identified in the homozygous state in exon 12, leads to the fall-out of the aminoacid Tyr402 in β1-domain of the protein of β-galactosidase, which has negative effect on the functional properties of the protein and may

a) p.Gly243Arg

species	aa	alignment																							
Human	243	A	L	Q	G	L	Y	T	T	V	D	F	G	T	G	S	N	I	T	D	A	F	L	S	Q
Our patient	**243**										**D**	**F**	**R**	**T**	**G**	**S**	**N**	**I**	**T**	**D**	**A**	**F**	**L**	**S**	
Ptroglodytes	243										D	F	**G**	T	G	S	N	I	T	D	A	F	L	S	
Mmusculus	244	T	L	Q	D	L	Y	A	T	V	D	F	**G**	T	G	N	N	I							
Ggallus	236	A	L	Q	G	L	Y	A	T	V	D	F	**A**	P	G	G	N								
Trubripes	237	A	M	Q	G	L	Y	A	T	V	D	F	**G**	P	G	S	N	V	T	A	A	F			
Drerio	262	S	L	Q	G	L	Y	A	T	V	D	F	**G**	P	G	A	N	V	T	A					
Dmelanogaster	236	K	I	Q	G	V	L	A	T	M	D	F	**G**	A	T	N	D	L	K	P					
Celegans	254	T	V	E	G	V	F	P	T	V	D	F	**G**	PTD	D	A	K	E	I	E	N	N	F	K	L
Xtropicalis	241	T	I	Q	G	L	Y	T	T	V	D	F	**G**		P	G	S	N	V	T					

c) p.Gly262Ala

species	aa	alignment																							
Human	262	A	F	L	S	Q	R	K	C	E	P	K	G	P	L	I	N	S	E	F	Y	T	G	W	L
Our patient	**262**	**A**	**F**	**L**	**S**	**Q**	**R**	**K**	**C**	**E**	**P**	**K**	**A**	**P**	**L**	**I**	**N**	**S**	**E**	**F**	**Y**	**T**	**G**	**W**	
Ptroglodytes	262	A	F	L	S	Q	R	K	C	E	P	K	**G**	P	L	I	N	S	E	F	Y	T	G	W	
Mmusculus	263	A	F	L	V	Q	R	K	F	E	P	K	**G**	P	L	I	N	S	E	F	Y	T	G	W	
Ggallus	255	A	F	L	A	Q	R	S	S	E	P	T	**G**	P	L	V	N	S	E	F	Y	T	G	W	
Trubripes	256			E	A	Q	R	H	A	E	P	R	**G**	P	L	V	N	S	E	F	Y	T	G	W	
Drerio	281	A	F	E	A	Q	R	H	V	E	P	R	**G**	P	L	V	N	S	E	F	Y	P	G	W	
Dmelanogaster	253	I	W	A	K	F	R	R	Y	Q	P	K	**G**	P	L	V	N	A	E	Y	Y	P	G	W	
Celegans	276						R	K	F	A	P	N	**G**	P	L	V	N	S	E	Y	Y	P	G	W	
Xtropicalis	260	T	F	S	V	Q	R	Y	C	E	P	K	**G**	P	L	V	N	S	E	F	Y	T	G	W	

Fig. 4. The comparison of a protein sequence encoded in the gene *GLB1* for a human protein and close homologs of mammals for the purpose of determining the conservative nature of aminoacid Gly in positions 243 and 262 (MutationTaster, PolyPhen2)

lead to the change in the splicing site (MutationTaster). This deletion was found in 2 alleles in combination with the missence replacement p.Pro403Tre, which may have no considerable impact on the protein properties (MutationTaster, PolyPhen2 and Provean).

The results of the prognostic analysis for the new missence mutations, identified by us, p.Gly243Arg (c.727G > A), p.Gly262Ala (c.785G > C), demonstrated that aminoacid Gly in both positions is rather conservative (Fig. 4), and its replacements, found in positions 243 and 262, have a damaging effect on the protein of β-galactosidase, which causes the destruction of its properties.

Another intron variant of the mutation c.1233+8T > C (IVS12+8T > C), found by us, was localized in the acceptor zone of the splicing site at the end of exon 12. This mutation leads to incorrect splicing of mRNA and, as a result, to the formation of abnormal protein product.

Four new mutations (c.699delG, c.833delG, Gly243Arg, p.Gly262Ala) were found in the patients with the infantile form of GM1-gangliosidosis in the heterozygous state in combination with other mutations, which, according to the data of scientific literature, cause the infantile form of this pathology (Table 3). The deletion c.699delG was also present in the genotype of one patient in the homozygous state, which resulted in very early onset of the disease. The bound inheritance of mutations c.1203_1205delTTA and p.Pro403Tre was identified in one patient in the homozygous state and conditioned the development of the infantile form of GM1-gangliosidosis. However, the mutation of the splicing site IVS12+8T > C, found by us, was identified in the patient with Morquio B disease in the heterozygous state in combination with the missence mutation p.Trp273Leu, common for this phenotype [13].

Thus, the spectrum of mutations in the gene *GLB1* in Ukrainian patients with GM1 gangliosidosis and Morquio B disease and their impact on the patients' phenotypes were studied in our work. Six new pathogenic mutations in the gene *GLB1* were identified with the characterization of their impact on the structure and function of the protein of β-galactosidase. The frequencies of alleles were determined and the major nature of the mutation p.His281Tyr (c.841C > T) for Ukrainian patients with GM1-gangliosidosis was defined. These results of our study provide the necessary information for more efficient and precise molecular diagnostics of the patients with GM1-gangliosidosis and Morquio B disease, for understanding the impact of mutations on the enzymatic activity which is responsible for the development of the disease. Also the results of molecular diagnostics are required to carry out prenatal diagnostics in high risk families, suffering from GM1-gangliosidosis and Morquio B disease.

Conclusions

The study of the spectrum of mutations in the gene *GLB1* in 26 Ukrainian patients with GM1-gangliosidosis and Morquio B disease allowed identifying 52 alleles, containing 17 types of pathogenic mutations.

The missence mutation p.His281Tyr (c.841C > T) has high incidence rate among Ukrainian patients with GM1-gangliosidosis (19/52 alleles) and may be considered to be the major mutation for this population.

The mutation p.Arg201His, which affects the development of the juvenile form of GM1-gangliosidosis, has dominating impact on the development of the patient's phenotype in case of combination with more severe mutations in the genotype, which are associated with the infantile form of the disease.

The mutation p.Trp273Leu is remarkable only for the patients with Morquio B disease, which testifies to the possibility of using the results of molecular and genetic analysis during the differential diagnostics of GM1-gangliosidosis and Morquio B disease.

The study allowed identifying six new pathogenic mutations, which had not been found in any electronic databases or previously described in scientific literature, including 3 deletions (c.699delG, c.833delG, c.1203_1205delTTA), 2 missence replacements (p. Gly243Arg, p.Gly262Ala) and 1 nucleotide replacement in the splicing site (IVS12+8T > C).

The results of the molecular and genetic analysis of mutations in gene the *GLB1* may be used for more precise molecular diagnostics of patients with GM1-gangliosidosis and Morquio B disease and prenatal diagnostics in the high risk families, suffering from this disease.

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Ідентифікація та характеристика шести нових мутацій в гені *GLB1* у пацієнтів з GM1-гангліозидозом та синдромом Моркіо В з України

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GM1-гангліозидоз (MIM# 230500) та мукополісахаридоз IVB (Моркіо В, MIM# 230500) аутосомно-рецесивні захворювання, що відносяться до лізосомних хвороб накопичення та викликані змінами в структурі одного й того ж гену – *GLB1*. Результатом мутацій в гені *GLB1* є дефіцит кислої β-галактозидази і, як наслідок, накопичення в лізосомах пацієнтів GM1-гангліозиду та кератансульфату. **Мета.** Проведення аналізу спектру мутацій в гені *GLB1* серед пацієнтів з GM1-гангліозидозом та синдромом Моркіо В в Україні. **Результати.** При проведенні молекулярного аналізу гену *GLB1* у 25 українських пацієнтів з діагнозом GM1-гангліозидоз та у 1 пацієнта з синдромом Моркіо В було проаналізовано 52 алелі, в яких виявлено 17 типів патогенних мутацій, серед яких 11 – місенс заміни, 3 – делеції, 1 – інсерція та 2 – мутації сайту слайсингу. Найчастішою виявилась місенс мутація p.His281Tyr (c.841C > T) у 8-му екзоні, яка була знайдена у 19 з 52 мутантних алелів (36,5 %) усіх обстежених пацієнтів. В ході дослідження було виявлено шість нових мутацій, які не були знайдені в електронних базах та не були описані раніше в літературі, серед яких 3 – делеції (c.699delG, c.833delG, c.1203_1205delTTA), 2 – місенс заміни (p.Gly243Arg, p.Gly262Ala) та 1 – заміна нуклеотиду в сайті сплайсингу (IVS12+8T > C). **Висновки.** Результати проведеного молекулярно-генетичного аналізу мутацій в гені *GLB1* можуть бути використані для більш точної молекулярної діагностики хворих з GM1-гангліозидозом та синдромом Моркіо В та пренатальної діагностики в сім'ях високого ризику обтяжених даним захворюванням.

Ключові слова: GM1-гангліозидоз, синдром Моркіо В, ген *GLB1*.

Идентификация и характеристика шести новых мутаций в гене *GLB1* у пациентов с GM1-ганглиозидозом и синдромом Моркио В с Украины

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GM1-ганглиозидоз (MIM # 230500) и мукополисахаридоз IVB (Моркио В, MIM # 230500) аутосомно-рецессивные заболевания, относящиеся к лизосомным болезням накопления и вызванные изменениями в структуре одного и того же гена - *GLB1*. Результатом мутаций в гене *GLB1* является дефицит кислой β-галактозидазы и, как следствие, накопление в лизосомах пациентов GM1-ганглиозида и кератансульфата. **Цель.** Проведение анализа спектра мутаций в гене *GLB1* среди пациентов с GM1-ганглиозидозом и синдромом Моркио В в Украине. **Результаты.** При проведении молекулярно-генетического анализа в гене *GLB1* у 26 пациентов с GM1-ганглиозидозом и синдромом Моркио В в Украине было выявлено 52 аллеля, содержащих патогенные мутации. Наиболее часто встречалась миссенс мутация p.His281Tyr (c.841C > T) в 8 экзоне, которая была найдена у 19 з 52 мутантных аллелей (36,5 %) всех обследованных пациентов. В процесе исследования было обнаружено шесть новых патогенных мутаций, которые не были найдены в электронных базах и не описаны ранее в литературе, среди которых 3 – делеции (c.699delG, c.833delG, c.1203_1205delTTA), 2 – миссенс замены (p.Gly243Arg, p .Gly262Ala) и 1 – замена нуклеотида в сайте сплайсинга (IVS12 + 8T > C). **Выводы.** Результаты проведенного молекулярно-генетического анализа мутаций в гене *GLB1* могут быть использованы для более точной молекулярной диагностики больных с GM1-ганглиозидозом и синдромом Моркио В и пренатальной диагностики в семьях высокого риска обремененных данным заболеванием.

Ключевые слова: GM1-ганглиозидоз, синдром Моркио В, ген *GLB1*.

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