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Molecular-genetic characterization of Ukrainian patients with mucopolysaccharidosis IIIA: identification of three new mutations in the heparan-N-sulfatase gene

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Mucopolysaccharidosis type III or Sanfilippo syndrome (MIM # 252900) is a rare hereditary autosomal-recessive metabolic disorder, which occurs due to the deficiency of heparan-N-sulfatase enzyme (EC 3.10.1.1). **Aim.** To identify the whole spectrum of mutations in *SGSH* gene in Ukrainian patients with MPS III A. **Methods.** RFLP-analysis, SSCP method, sequencing. **Results.** We have identified 100 % (42/42) mutant alleles of *SGSH* gene in 23 patients (two probands had siblings with identical genotypes) with MPS III A from 21 Ukrainian family. The range of mutations in *SGSH* gene in Ukrainian patients with MPS III A is represented with 7 known missence-mutations , R74C, R245H, T271M, E292K, S298P, E369K, N389K and 2 single nucleotide deletions, c.1080delC and c.1135delG. We identified three new mutations in the *SGSH* gene: a missence-mutation G149R,a deletion c.216delC, and a deletion of 27 bp TCC^348CTCctgccggcgctggaggccg agcccctcTGGGCCACC. **Conclusions.** The data obtained may be useful for molecular-genetic analysis of Ukrainian patients with MPS III A.

K e y w o r d s: mucopolysaccharidosis, Sanfilippo A syndrome, heparan-N-sulfatase.

Introduction

Mucopolysaccharidosis type III (MPS III), also called Sanfilippo syndrome, is a rare hereditary autosomal-recessive metabolic disorder, which occurs due to the deficiency of one out of four lysosomal enzymes, involved in the multistage catabolism of heparan sulfate (HS). There are four biochemical subtypes of MPS III (A, B, C, D), caused by the deficiency of a specific enzyme: heparan–N–sulfatase (EC 3.10.1.1, causing MPS III A; MIM # 252900); α–N–acetylglucosaminidase (EC 3.2.1.50, causing MPS III B; MIM # 252920); acetyl–CoA: α-glucosaminide–N–acetyltransferase (EC 2.3.1.78, causing MPS III C; MIM # 252930), and N–acetylglucosamine–6–sulfatase (EC 3.1.6.14, causing MPS III D; MIM # 252940).

A decrease in the activity of any of these enzymes causes the accumulation of HS in the lysosomes of cells with subsequent excretion of the excess of this polysaccharide with biological liquids of the organism, thus resulting in the expressed degeneration of the central nervous system with progressing mental retardation and pathology of the connective tissue with some somatic and skeletal manifestations [1, 2].

Subtype III A is the most common for Northern Europe (with the approximate frequency of 1: 150 000) and the most severe disorder among the types of mucopolysaccharidosis III with the onset in

the early age, rapid progression of symptoms and a shorter life span [3-7].

The human heparan-N-sulfatase gene (*SGSH)* is localized in locus 17q25.3; it is 11 thousand bp long, consists of 8 exons [8, 9], and controls the synthesis of a protein, consisting of 502 aminoacids and containing 5 N-glycosylated sites. At present there are over 120 identified and characterized mutations in the *SGSH* gene, including missence\nonsense-mutations, small deletions and insertions, two large deletions and two splicing mutations (Human Gene Mutation Database-HGMD, http://www.hgmd.cf.ac.uk/ac/index.php). The missence-mutations R74C and R245H are the major for European populations, as their total frequency varies from 56 % in Germany, Poland, and the Netherlands to 33 % in Italy [10-12]. According to the data of our previous studies, in Ukraine the total frequency of these major mutations in *SGSH* gene is 64.58 % [13], other kinds of mutations in this gene in patients with MPS III A have not been estimated.

The aim of our work was to identify the whole spectrum of mutations in the *SGSH* gene in Ukrainian patients with MPS III A.

Materials and Methods

The material of the research was the biological material (blood) of the patients with MPS III A (23 children from 21 family), whose diagnosis was confirmed by biochemical methods with the determination of the activity of heparan-N-sulfatase in lysosomes, as well as of their 47 relatives (siblings and parents) from different regions of Ukraine [13, 14]. The age of patients at the moment of the diagnosis determination was 5 ± 1 years; there were 15 boys and 8 girls. Two children had siblings with identical genotypes, thus, the genetic profiles of 21 patients with MPS III A were further analyzed. The parents of all the patients gave their informed consent for the research. The Bioethics Committee approved the decision about the possibility of conducting the research.

DNA was extracted from the whole blood using the commercial kits NeoSorb (Neogen, Ukraine). The identification of major mutations R74C and R245H was conducted by the RFLP-analysis, as described in our publication [13]. For R74C detection: forward primer5'-TCACAGTCCCAGCCTCCCTACTCC-3', reverse primer 5'-CCCGGGATCCCAGGGATGGG AGAC-3', digestion by BstFNI. For R245H detection: forward primer 5'-GGCTAACCCATTTGCAG GAGGCCC-3', reverse primer 5'-CTCACCCACAT TATGCCGTGACCT-3', digestion by EagI. The primary screening of other mutations was conducted by the single strand conformation polymorphism method (SSCP) with SYBR®Gold (Life technologies, USA) staining following the manufacturer's protocol [15, 16]. The confirmation of the availability of mutations in the *SGSH* gene was performed by the Sanger's method of direct automated sequencing using ABI 3130 analyzer (Applied Biosystems) following the manufacturer's protocol. Primer sequences for each of eight exons of [the] *SGSH* gene were selected using Primer3 program (http://simgene.com/Primer3). The analysis of the results was made using the programs Sequencing Analysis, v.1.1/3.1 (Applied Biosystems, Life Technologies Corporation, USA), Chromas and Blast (http://www.ncbi.nlm.nih.gov/blast). To exclude polymorphic character of new rearrangements in the *SGSH* gene the following methods were used: for deletion c.216delC – RFLP analysis with BseYI, for deletion 27 bp – the capillary electrophoresis using «MultiNA» analyzer (Shimadzu biotech, Japan). The analysis of the pathogenicity of new mutations was made using programs PolyPhen2 and Provean (http://genetics.bwh.harvard.edu/pph2/, http://provean.jcvi.org/index.php).

Results and Discussion

Our previous work highlighted the screening results of the major mutations R74C and R245H in the *SGSH* gene in Ukrainian patients with MPS III A [13], which demonstrated that the share of alleles, carrying the mutation R74C, was the highest – 27/42 (64.3 %) (Table 1). Nine patients were homozygous carriers of this allele and nine – heterozygous carriers of this mutation. The mutation R245H in exon six of the *SGSH* gene was found in one patient only (2.4 %), who was defined as a compound heterozygote R74C/ R245H.

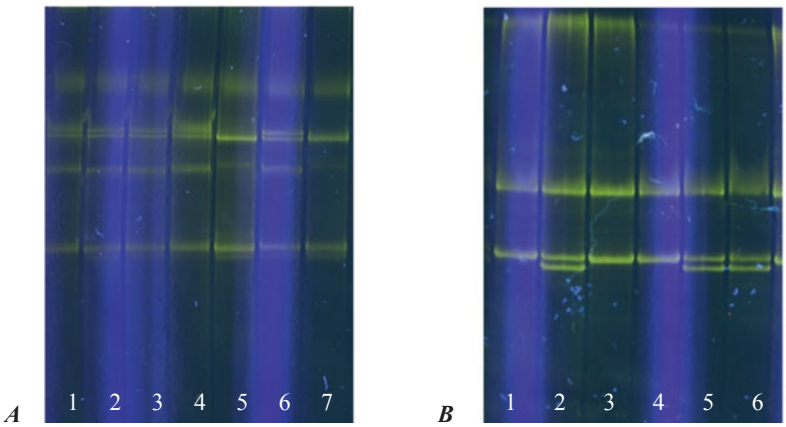

Fig. 1. The results of SSCP-analysis for fragments of exons 2 and 8, *SGSH* gene:
A – the electrophoregram of exon 2:
1 – control sample of a healthy person;
2,3,4,6 – samples with normal DNA fragments;
5,7 – samples with abnormal DNA fragments;
B – the electrophoregram of exon 8:
1 – control sample of a healthy person;
3,4 – samples with normal DNA fragments;
2,5,6 – samples with abnormal DNA fragments.

According to the recent publications of molecular-genetic studies of the characteristics of patients with MPS III A in European populations, the highest amount of mutations in the *SGSH* gene is localized in exons 2, 4, 5, 7, and 8 [17]. Our screening of all eight exons of the *SGSH* gene by SSCP methods in the patients, their parents, and in the control samples of healthy individuals was conducted to identify the abnormal conformation of DNA fragments during their refolding. The results obtained demonstrated that the highest number of abnormal DNA fragments of patients, which differed from the DNA fragments of healthy individuals, was found in exons 2, 7 and 8 of the *SGSH* gene, which is in good agreement with the published data about the highest number of mutations in these exons [17].

Our final identification of the mutations in the *SGSH* gene involved the method of direct automated sequencing of the exons, intron sites, and flanking non-coding regions of the *SGSH* gene. Firstly, the exons, marked as "hot" ones according to the primary screening results by SSCP method, were studied. Notably, the mutation R74C, major for both most European populations and Ukraine, is localized in the second exon of the *SGSH* gene, which conditioned a high number of abnormal DNA fragments in this exon (Fig. 1).

Overall, our studies allowed identifying 100 % (42/42) of mutant alleles of the *SGSH* gene. The complete molecular-genetic characteristic of the patients with MPS III A, with the consideration of the severity degree of the clinical course, is presented in Table 1.

Therefore, the spectrum of mutations in the *SGSH* gene in the Ukrainian patients with MPS III A is represented with 8 missence-mutations, 3 single nucleotide deletions, and a large deletion of 27 bp (Fig. 2).

Five previously described mutations in the exons 7 and 8 - T271M, E292K, S298P, E369K, N389K – were detected among missence-mutations, identified during the analysis in addition to major mutations R74C and R245H, the presence of which in Ukrainian patients with MPS III A was analyzed in the previous work [13] (Table 2). The described single nucleotide deletion c.1080delC in exon 7 was identified in one patient, in one allele. The deletion c.1135delG, previously described in exon 8, was identified in two patients.

Additionally, three mutations in the *SGSH* gene, not described previously, were found, including one missence-mutation G149R in exon 4, the deletion of one nucleotide in exon 2 c.216delC, and deletion 27 bp TCC^348CTCctgccggcgctggaggccgagcccctcT GGGCCACC in exon 8 (Fig. 3). The restriction fragment length polymorphism analysis of c.216delC deletion and analysis of electrophoretic mobility of the 8th exon of the *SGSH* gene in 100 alleles of healthy individuals did not reveal any similar rearrangements, thus, these new mutations may not be referred to polymorphisms. The detailed analysis of these mutations using PolyPhen2 and Provean programs also confirmed their pathological character (Fig. 3).

Therefore, two mutations may be considered as the major ones for the Ukrainian patients with MPS III A: R74C (64.3 %) and N389K (7.8 %). Other mu-

Table 1. **The results of molecular and genetic analysis of Ukrainian patients with MPS III A**

Patients	Gender	Age at the moment of diagnosis determination	Severity degree	Nucleotide replacement in *SGSH* gene	Aminoacid replacement in the molecule of heparan-N-sulfatase	Reference to the first description of mutation in *SGSH* gene
1	F	4.5 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.1080delC	p.Val361SerfsX52	Weber *et al,* 1997
2	M	9 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
3	M	11 y.o.	Medium	c.220C>T	p.R74C	Weber *et al,* 1997
				c.892T>C	p.S298P	Bunge *et al,* 1999
4	F	5 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
5	M	6 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
6	F	4 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
7	F	4 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.1135delG	p.Val379fsX33	Muschol *et al,* 2004
8	M	6.5 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
9	M	11 months	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
10	M	4 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
11	M	3.5 y.o.	Medium	c.220C>T	p.R74C	Weber *et al,* 1997
				c.1167C>A	p.N389K	Bunge *et al,* 1999
12	M	6 y.o.	Severe	c.812C>T	p.T271M	Heron *et al,* 2010
				c.1135delG	p.Val379fsX33	Muschol *et al,* 2004
13	F	3 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.734G>A	p.R245H	Blanch *et al,* 1997
14	F	6 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
15	F	6 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.1105G>A	p.E369K	Di Natale *et al,* 1998
16	F	3.5 y.o.	Medium	c.1105G>A	p.E369K	Di Natale *et al,* 1998
				c.1167C>A	p.N389K	Bunge *et al,* 1999
17	M	2 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.220C>T	p.R74C	Weber *et al,* 1997
18	M	8 y.o.	Medium	c.220C>T	p.R74C	Weber *et al,* 1997
				c.874G>A	p.E292K	Piotrowska *et al,* 2009
19	M	5 y.o.	Severe	c.216delC	p.Pro72fsX244	Our studies
				c.1167C>A	p.N389K	Bunge *et al,* 1999

Patients	Gender	Age at the moment of diagnosis determination	Severity degree	Nucleotide replacement in *SGSH* gene	Aminoacid replacement in the molecule of heparan-N-sulfatase	Reference to the first description of mutation in *SGSH* gene
20	M	2.5 y.o.	Medium	c.220C>T	p.R74C	Weber *et al,* 1997
				c.1045_1070 delCTGCCGGCG CTGGAGGCCGAGCCCCTC	p.Leu348fsX146	Our studies
21	M	5.5 y.o.	Severe	c.220C>T	p.R74C	Weber *et al,* 1997
				c.446G>A	p.G149R	Our studies

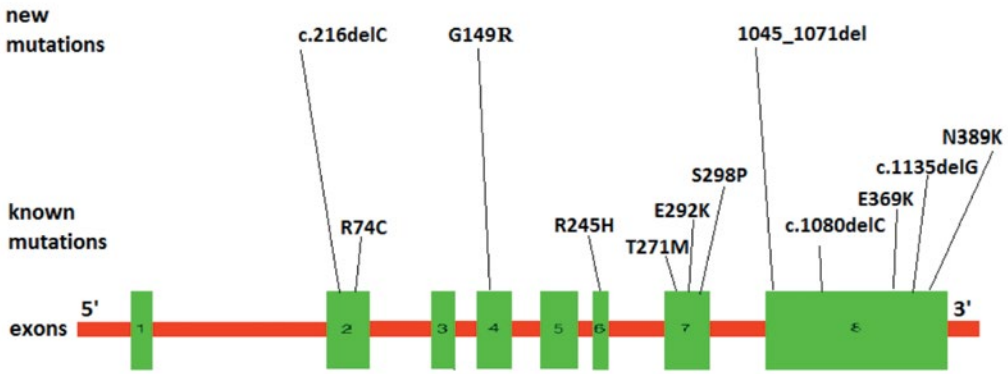

Fig. 2. The localization of mutations in specific exons of the *SGSH* gene in Ukrainian patients with MPS III A

Table 2. **The localization of identified mutations by exons of *SGSH* gene and their frequency**

No	Mutation	Number of exon	Number of mutant alleles in percentage ratio	Reference
1	c.216delC	2	2.4 % (1/42)	New mutation
2	p.R74C	2	64.3 % (27/42)	[10]
3	p.G149R	4	2.4 % (1/42)	New mutation
4	p.R245H	6	2.4 % (1/42)	[20]
5	p.T271M	7	2.4 % (1/42)	[19]
6	p.E292K	7	2.4 % (1/42)	[22]
7	p.S298P	7	2.4 % (1/42)	[11]
8	c.1045_1070del CTGCCGGCGCTGGAGGCCGAGCCCCTC	8	2.4 % (1/42)	New mutation
9	c.1080delC	8	2.4 % (1/42)	[10]
10	p.E369K	8	4.8 % (2/42)	[21]
11	c.1135delG	8	4.8 % (2/42)	[18]
12	p.N389K	8	7.1 % (3/42)	[11]

tations are represented in single cases. These frequencies may change with the further identification of new patients. Most rare mutations, identified by us in the Ukrainian patients with MPS III A, were localized in exons 7 and 8, and one – in exons 2 and 4 of the *SGSH* gene.

The studies of genotype-phenotype correlations for MPS III A, conducted in different countries, are based on the division of all the patients into two phenotypic groups: severe (with early onset and severe clinical course of the disease), and medium (with late onset and a milder phenotype) [23].

The results of the molecular-genetic analysis of the Ukrainian patients with MPS III A demonstrated that all the patients with the major mutation R74C in the homozygous state, and a patient, who was identi-

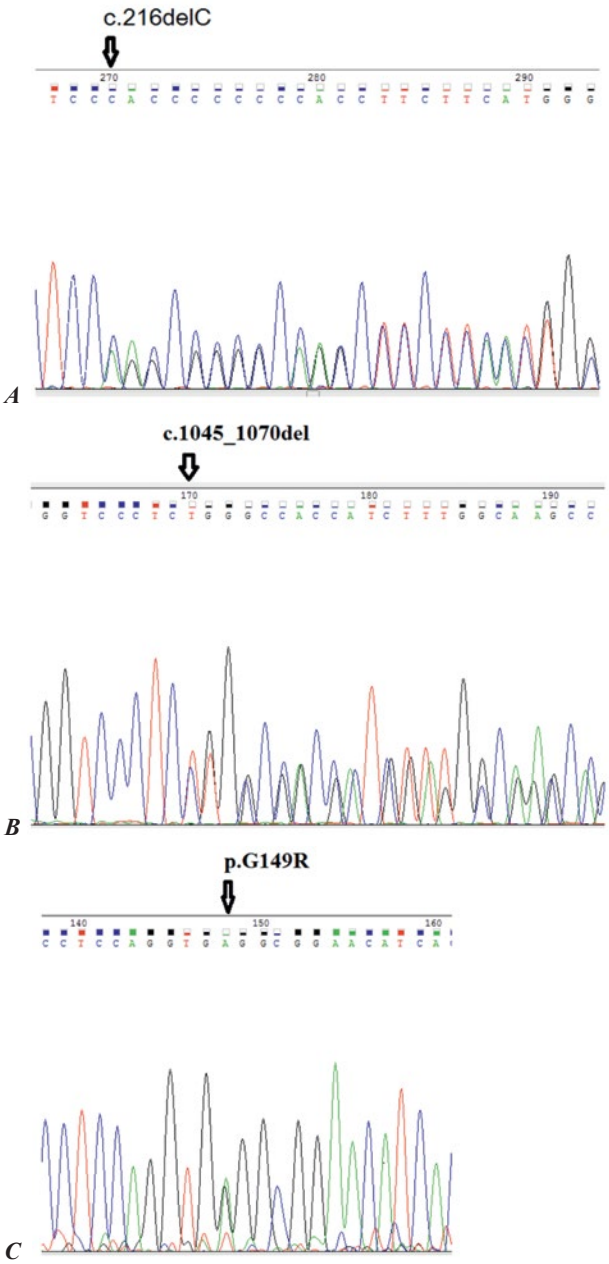

Fig. 3. The chromatograms of the DNA sequence of new mutations in *SGSH* gene: *A* – deletion c.216delC in exon 2; *B* – deletion c.1045_1070delCTGCCGGCGCTGGAGGCCGAGCCCC TC in exon 8; *C* – missence-mutation G149R in exon 4.

fied as a compound heterozygote R74C/ R245H, had a severe form of the disease, which is in agreement with the data of other researchers about the association of major missence-mutations R74C and R245H with the severe clinical course [18]. The severe clinical course was also remarkable for patients with the major mutation R74C in one allele in single nucleotide deletions c.1080delC (1 person) and c.1135delG (2 siblings) and the missence-mutation E369K (1 person), which is in agreement with the previous data about the association of these mutations with early onset of the disease [10, 18]. This group also includes the patients with a single nucleotide deletion c.1135delG in the compound with the missence-mutation T271M.

It was demonstrated in several works that the missence-mutation S298P often has a mitigating effect on the phenotype, even with a "severe" mutation in another allele [24]. Among the patients, studied by us, the mentioned mutation was identified in one person in the compound with the major mutation R74C, when it conditioned the development of a mild form of the disease with late onset. Some mitigating effect of the phenotype is also noted for the missence-mutations N389K and E292K, which conditioned medium-severe clinical course in the compound with "severe" R74C and E369K in three studied patients.

The patient with the compound combination of the major mutation R74C and the substitution G149R, previously described by us, as well as the patient with genotype c.1167C>A /c.216delC were characterized with severe phenotype and early onset of the disease, while the patient with the mutation R74C in one allele and the deletion of 27 bp in another was remarkable for a milder phenotype and medium-severe clinical course.

Therefore, our analysis of molecular-genetic characteristics of Ukrainian patients with MPS III A identified 100 % mutant alleles. High incidence of the mutation R74C (64.3 %) among the patients from Ukraine substantiates the conducting of RFLP-screening at the first stage of the molecular-genetic analysis. The mutation R245H, very common in some European populations, was found only in one allele in the examined patients, thus RFLP-screening for it is not reasonable for our country. It would be feasible to start the identification of rare mutations, which conditioned the development of the disease in the proband, with "hot"

regions of the *SGSH* gene – exons 2, 7 and 8, where the most pathogenic mutations were localized in the examined patients. This order of analysis planning would allow optimizing both time and resources for the analysis and ensuring the maximal efficiency of the molecular-genetic diagnostics of MPS III A in Ukraine.

Conclusions

100 % mutant alleles were identified while conducting the molecular-genetic analysis of pathogenic mutations in the *SGSH* gene in 23 patients with MPS III A from 21 families (42 alleles) in Ukraine.

The share of the alleles, carrying the major mutation R74C, was the highest – 64.3 % (27/42). The second mutation, major for European populations, R245H, was identified only in one patient (2.4 %), in one allele, thus, it cannot be deemed major for Ukraine.

Most rare mutations, identified by us in Ukrainian patients with MPS III A, are localized in exons 7 and 8 of the *SGSH* gene and one for exons 2 and 4.

Three previously not described mutations were identified in the *SGSH* gene – c.216delC, c.446G>A and c.1045_1070delCTGCCGGCGCTGGAGGCCGAGCCCCTC.

The data of the conducted molecular-genetic analysis of the mutations in the *SGSH* gene may be used to plan a more reasonable algorithm of the molecular-genetic analysis for Ukrainian patients with MPS III A in order to enhance the quality of medical services for the families, suffering from this pathology.

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Молекулярно-генетична характеристика пацієнтів з мукополісахаридозом ІІІ А з України: виявлення трьох нових мутацій в гені гепаран-N-сульфатази

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Мукополісахаридоз ІІІ А типу або синдром Санфіліппо А (MIM # 252900) – рідкісне спадкове аутосомно-рецесивне метаболічне захворювання, яке виникає внаслідок дефекту ферменту гепаран–N–сульфатази (ЕС 3.10.1.1). **Мета.** Виявлення повного спектру мутацій в гені *SGSH* у пацієнтів з МПС ІІІ А з України. **Методи.** ПДРФ-аналіз, метод SSCP, метод прямого автоматичного секвенування по Сенгеру на аналізаторі ABI 3130 (Applied Biosystems). **Результати.** На підставі проведених нами досліджень було ідентифіковано 100 % (42/42) мутантних алелів гену *SGSH* серед 23 хворих (два пробанди мали сібсів з ідентичними генотипами) на МПС ІІІ А пацієнтів з 21 родини з України. Спектр мутацій в гені *SGSH* у пацієнтів з МПС ІІІА в Україні представлений відомими 7 місенс-мутаціями – R74C, R245H, T271M, E292K, S298P, E369K, N389K та 2 однонуклеотидними делеціями – c.1080delC і c.1135delG. Нами було знайдено три нових мутації в гені *SGSH*: місенс-мутація G149R, делеція c.216delC, та делеція 27 п.н. TCC^348 CTCctgccggcgctggaggccgagcccctcTGGGCCACC. **Висновки.** Дані проведеного молекулярно-генетичного аналізу мутацій в гені *SGSH* можуть бути використані при плануванні найбільш раціонального алгоритму молекулярно-генетичного аналізу пацієнтів з МПС ІІІА в Україні.

Ключові слова: мукополісахаридоз, синдром Санфіліппо А, гепаран-N-сульфатаза.

Молекулярно-генетическая характеристика пациентов с мукополисахаридозом ІІІ А из Украины: виявление трёх новых мутаций в гене гепаран-N-сульфатазы

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Мукополисахаридоз ІІІ А типа или синдром Санфилиппо А (MIM # 252900) – редкое наследственное аутосомно-рецессивное метаболическое заболевание, которое возникает вследствие дефекта фермента гепаран-N-сульфатазы (ЕС 3.10.1.1). **Цель.** Выявление полного спектра мутаций в гене *SGSH* у пациентов с МПС ІІІ А из Украины. **Методы.** ПДРФ-анализ, метод SSCP, метод прямого автоматического секвенирования по Сенгеру на анализаторе ABI 3130 (Applied Biosystems). **Результаты.** На основании проведенных нами исследований было идентифицировано 100 % (42/42) мутантных аллелей гена *SGSH* среди 23 пациентов (два пробанда имели сибсов с идентичными генотипами) с МПС ІІІ А из 21 семьи из Украины. Спектр мутаций в гене *SGSH* у пациентов с МПС ІІІ А в Украине представлен известными 7 миссенс-мутациями – R74C, R245H, T271M, E292K, S298P, E369K, N389K и 2 однонуклеотидных делециями – c.1080delC и c.1135delG. Нами было найдено три новых мутации в гене *SGSH*: миссенс-мутация G149R, делеция c.216delC, и делеция 27 п.н. TCC^348CTC ctgccggcgctggaggccgagcccctcTGGGCCACC. **Выводы.** Данные проведенного молекулярно-генетического анализа мутаций в гене *SGSH* могут быть использованы при планировании наиболее рационального алгоритма молекулярно-генетического анализа пациентов с МПС ІІІ А в Украине.

Ключевые слова: мукополисахаридоз, синдром Санфилиппо А, гепаран-N-сульфатаза.

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