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Genetics of Mucopolysaccharidosis Type IV (Morquio Disorder) in Patients from Azerbaijan

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Genetic screening in the Azerbaijan Republic for mucopolysaccharidosis disorder has been implemented.

The purpose of the work was to study types of mucopolysaccharidosis mutations and discuss ways of disorder prophylaxis in the family with parents of reproductive ages.

Materials and methods. Material for studies was collected in the specialized children medical centers in Baku city, Azerbaijan, as well as in the field works in the regions of the Republic for 2018–2022. Patients were chosen during clinical examinations by pediatrician and geneticist. To screen mucopolysaccharidosis disorder a complex of modern molecular-genetic diagnostics methods have been applied. 56 patients were identified in the age varied between six months and 28 years. Gender differentiation was as follows: 15 males and 11 females. Blood sampling was done onto dry blood sample cards. All patients have undergone enzyme analysis for all mucopolysaccharidosis types.

Results and discussion. The carried-out screening of enzymatic analysis allowed us to identify 26 patients with the N-acetylgalactosamine-6-sulfat sulfatase enzyme deficit out of disorder suspicious 56 persons. And that was specific for mucopolysaccharidosis type IV A. That counted 46.4% of all studied patients. Seven mutation types in homozygous, double heterozygous (compound) and heterozygous state were identified. All mutations have nucleotide substitution. Practical application of the results is being discussed. Mucopolysaccharidosis type IV frequency was higher than other mucopolysaccharidosis disorder types. In eight patients the level of the enzyme was very low and varied between <0.1 (LOD) μmol/L/h and <0.3 (LOD) μmol/L/h, which is specific for homozygous or double heterozygous state, when norm is ≥2.0 mol/L/h. In 18 patients the activity level of N-acetylgalactosamine-6-sulfate sulfatase enzyme was almost half reduced (<0.6 (LOD) μmol/L/h - <0.1 (LOD)), which speaks to heterozygous state of disorder.

Conclusion. Thus, for the first time populational study of mucopolysaccharidosis disorder by means of molecular-genetic modern complex has been carried out. Molecular-genetic analysis allowed our identification of 7 GALNS gene mutation types: 553 C>T, 439 T>A, 1283 A>G, 157 G>A, 463 G-T, 1018 G-T and 443 A>G. These mutations have nucleotide substitutions and have been priory described in references.

Keywords: storage disorder, N-acetylgalactosamine-6-sulfate sulfatase, β-galactosidase, MPS IVA enzyme, gene, mucopolysaccharidosis, autosomal-recessive inheritance type.

Introduction. Mucopolysaccharidoses (MPS) is a group of inherited metabolic disorders linked with metabolism damage of glycosaminoglycans (GAG) leading to human organs and tissues.

Synonyms for mucopolysaccharidosis type IV (MPS IV) are Morquio disorder, spondylo-epiphyseal dysplasia, chondrodystrophy, deforming osteochondrodistrophy, Morquio-Brilesford syndrome, Morquio-Ulrich syndrome, K-mucopolysaccharidosis, ex-centrochondroplasia, Dugve-Melchior-Clausen syndrome. Disorder was described in 1929 by Uruguayan pediatrician Luis Morquio (1867–1935) and James Frederick Brailsford (1888–1961), English radiologist in Birmingham, England [1–5].

The said storage disorder was determined with lysosomal hydrolase deficiency: N-acetylgalactosamine-6-sulfate sulfatase (MPS IVA) or β-galactosidase (MPS IVB) which is specifically storing keratan sulfate in connective tissues and is characterized by significant skeletal deformation and stunted growth. All above shown traits could lead a patient to disability and with severe course of disorder even to lethal end. Being different from other mucopolysaccharidoses types, type IV is indicated with no intelligence loss, no retina “cloudy” damage, mild hepato-splenomegaly and mildly “coarse” face features. Several forms of MPS type IV are known as severe or classical, intermediate and light [6–8].

N-acetylgalactosamine-6-sulfatase (GALNS) is mapped on Chromosome 16 (locus 16q 24.3) [8].

Full-size cDNA of human N-acetylgalactosamine-6-sulfate sulfatase is cloned and sequenced: encoding sequence 1566 nucleotides ES coding a polypeptide off 522 amino acids consistent of 26 amino acids of the signal peptide and 495 ami-

no acids of the mature peptide. Propeptide with molecular weight off 60 kDa forms two peptides off 40 kilodalton and 15 kDa as a result of proteolytic splice. Length of GALNS gene is 15 kb and consists of 14 exons and 13 introns in between the size of exons varies between 75 and 790 nucleotides. The intron size could be from 380 to 14000 nucleotides. 5' Flanking area of GALNS gene losses canonic "TATA-box" and "CCAAT-box" – sequences. Instead of them promoter area carries 4 inverted (antisense) "GC-box" (CCG-CCC), 5 (sense) "GC-box" (GGGCGG) sequences, linking transcription factors' sites Sp1. Polyadenyling signal AATAAA locates in exon 14 after 33 nucleotides from Poly-A site. In introns 5 and 6 there Alu repetitions and VNTR-like sequences, unique mRNA 2.3 kb long is identified in cells [9–11].

Morquio syndrome is one of the rarest kinds of mucopolysaccharidoses. There is no exact data, but the disorder is encountered as around 1 out of 200,000 to 300,000 live births and nevertheless, it is a very rare disorder, but every single patient needs in very wide and multivector medical help. So, the MPS influence on the health management should be the way wider than it could be prognosed according to statistics numbers [12–13].

In most countries of the World the epidemiological work has been carried out for identification of MPS disorders in different population groups. In the US the frequency of all types MPS disorders was counted as 0.98 in 100,000 live births, in Poland – 1.8 for 100,000, in Japan and Switzerland – 1.53 for 100,000. In Switzerland that was 1.56 for 100,000 live births [14–23].

Inheritance type for MPS type IV is autosomal recessive [19].

Our study on kid-patients identification and diagnosed MPS, especially MPS IV has been the first study for the Azerbaijan Republic population. At the same time firstly for patients' identification with modern molecular-genetic methods we plan testing GALNS gene mutations and frequency statistics for this given type among the entire MPS patient numbers.

The purpose of the work was to study types of mucopolysaccharidosis mutations and discuss ways of disorder prophylaxis in the family with parents of reproductive ages.

Materials and methods. Since 2018 and up to now material works were collected in special children's medical institutions in Baku city as well as in field expeditions to 2 regions of the Republic. Patients were chosen during clinical examination by pediatricians and geneticists. 56 patients were identified in the age varied between six months and 28 years of age. Gender differentiation was as follows: 15 males and 11 females. Blood sampling was done onto DBS (dry blood sample) cards.

The study was carried out in compliance with the basic provisions of the "Rules of ethical principles of scientific medical research with human participation", approved by the Declaration of Helsinki (1964-2013), ICH GCP (1996), EEC Directive No. 609 (dated 24.11.1986), Orders of the Ministry of Health of Ukraine No. 690 (dated 23.09.2009), No. 944 (dated 14.12.2009), No. 616 (dated 03.08.2012). Parents of each study patient signed an informed consent to participate in the study and all measures to ensure anonymity of patients were taken.

All patients have undergone enzyme analysis for all MPS types. For that reason, the following enzymes were used: α-L-iduronidase (MPS I), iduronat-2-sulfatase (MPS II), heparan-N-sulfatase (MPS IIIA), N-acetylglucosaminidase (MPS IIIB), N-acetyltransferase (MPS IIIC), N-acetylglucosamine 6-sulfatase (MPS IIID), N-acetylgalactosamine-6-sulfate sulfatase (MPS IVA), β-galactosidase (MPS IVB), arylsulfatase B (MPS VIB).

Fluorometry method was applied in 4 identification of enzyme activity and mutation testing was carried out with NGS method.

GALNS gene in DNA sample obtained from peripheral blood was studied by new generation sequencing method. More than 99% coding regions of those genes have been studied with reading depth no less than 50X. Mean reading depth consists of 1,559 indications. Connections exon-intron (± 10np) were included into analysis. Obtained data pathogeny classification was carried out according to «Guidelines of ACMG*».

Results and discussion. 26 out of 56 patients have shown MPS IV disorder as a result of carried-out screening, using enzymes for all MPS types to exact identification of the N-acetylgalactosamine-6-sulfate sulfatase enzyme deficit, which is specific for MPS IVA.

In eight patients the level of the enzyme was very low and varied between <0.1 (LOD) μmol/L/h and <0.3 (LOD) μmol/L/h, which is specific for homozygous or double heterozygous state, when norm is ≥2.0 mol/L/h.

In 18 patients the activity level of N-acetylgalactosamine-6-sulfate sulfatase enzyme was almost half reduced (<0.6 (LOD) μmol/L/h – <0.1 (LOD)), which speaks to heterozygous state of disorder.

N-acetylgalactosamine-6-sulfat sulfatase enzyme analysis results for seven patients suspicious with disorder are presented in **Table 1**.

In 26-year-old patient Sh.S., low activity level of two enzymes was observed: arylsulfatase B – 8.0 μmol/L/h (N ≥8.8 μmol/L/h) and N-acetylgalactosamine-6-sulfat sulfatase – 1.3 μmol/L/h (N ≥2.0 μmol/L/h). These data are presented in **Table 2**.

Table 1 – N-acetylgalactosamine-6-sulfat sulfatase enzyme level analysis results in patients with MPS IVA

Patient	Result	Reference	Zygosity	Interpretation	Method
A.S.	<0.3 (LOQ) μmol/L/h LOQ = limit of quantification	≥2.0 mol/L/h	Homozygote	Pathologic	Liquid chromatography mass spectrometry
V.A.	<0.1 (LOD) μmol/L/h LOD = limit of detection	≥2.0 μmol/L/h	Homozygote	Pathologic	Liquid chromatography mass spectrometry
V.G.	<0.1 (LOD) μmol/L/h LOD = limit of detection	≥2.0 μmol/L/h	Homozygote	Pathologic	Liquid chromatography mass spectrometry
A.A.	<0.1 (LOD) μmol/L/h	≥2.0 μmol/L/h	Homozygote	Pathologic	Liquid chromatography mass spectrometry
G.N.	<0.1 μmol/L/h	≥2.0 μmol/L/h	Homozygote	Pathologic	Liquid chromatography mass spectrometry
A.M.	<0.1 (LOD) μmol/L/h LOD = limit of detection	≥2.0 μmol/L/h	Homozygote	Pathologic	Liquid chromatography mass spectrometry
A.N.	< 0,1 (LOD) μmol/L/h LOD = limit of detection	≥2.0 μmol/L/h	Homozygote	Pathologic	Liquid chromatography mass spectrometry

Table 2 – Patient Sh. S. enzymes analysis results

Name of gene / enzyme / biomarker	Result	Reference	Interpretation	Method
Alpha-iduronidase	3.1 μmol/L/h	≥3.0 μmol/L/h	Normal	Fluorimetry
Arylsulfatase B	8.0 μmol/L/h	≥8.8 μmol/L/h Pathologic	Pathologic*	Fluorimetry
ARSB	No clinically relevant variant	NM_000046.3	Normal	NGS - illumina
Beta-galactosidase	90.4 μmol/L/h	28.5 μmol/L/h	Normal	Liquid chromatography mass spectrometry
N-acetylgalactosamine-6-sulfate sulfatase	1.3 μmol/L/h	≥2.0 μmol/L/h	Pathologic	Liquid chromatography mass spectrometry

All 26 patients with abnormal N-acetylgalactosamine-6-sulfate sulfatase enzyme activity have undergone genetic analysis for GALNS gene. Results of those genetic analyses on GALNS gene for 8 patients suspicious to have homozygous state are presented in **Table 3**.

Patient A.S. showed a mutation: substitution of Cytosine nucleotide with Thymine nucleotide in position 553 of GALNS gene as homozygous state. Because of that the GALNS variant c. 553 C>T p. (Pro185Ser) causes an amino acid change from Pro to Ser at position 185. According to HGMD Professional 2019.1, this variant has previously been described as disease causing Mucopolysaccharidosis IVA by Terzioglu et. al., 2002 (PMID: 12442278), Rivera-Colon et. al., 2012 (PMID: 22940367).

It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG. Pathogenic variants in GALNS gene are associated with Mucopolysaccharidosis type IVA, an autosomal recessive disorder. Mucopolysaccharidosis type IV (MPS IV) is a lysosomal storage disease belonging to the group of Mucopolysaccharidoses and characterized by spondylo-epiphyso-metaphyseal dysplasia. It exists in two forms: A and B. MPS IVA is a spondylo-epiphyso-metaphyseal dysplasia generally

Table 3 – Genetic analysis results of N-acetylgalactosamine-6-sulfate sulfatase gene

Patient	Variant coordinates	Amino acid change	Zygosity
A.S.	NM_001323544.1: c. 553 C>T	p. (Pro185Ser)	Homozygous
V.A.	NM_001323544.1: c. 439 T>A	p. (Trp147Arg)	Homozygous
V.G.	NM_001323544.1: c. 1283 A>G	p. (Gln428Arg)	Homozygous
G.N.	NM_001323544.1: c. 157 G>A NM_001323544.1: c. 553 C>T	p. (Gly53Arg) p. (Pro185Ser)	Heterozygous Heterozygous
A.A.	NM_001323544.1: c. 439 T>A	p. (Trp147Arg)	Heterozygous
A.M.	ENSG00000141012NM - 001323543 c, 463 G-T ENSG00000141012/ENST00000268695 c, 1018 G-T	p. (Gly133Cys) p. (Gly340Cys)	Heterozygous Heterozygous
A.N.	ENSG00000141012NM-001323543 c, 463 G-T ENSG00000141012/ENST00000268695 c, 1018 G-T	p. (Gly133Cys) p. (Gly340Cys)	Heterozygous Heterozygous

diagnosed during the second year of life, after walking acquisition. Skeletal deformities (platyspondyly, kyphosis, scoliosis, pectus carinatum, genu valgum, long bone deformities) become more pronounced as the child grows. Joint hyperlaxity is accompanied by frequent luxation (hips, knees).

Homozygous state mutation: substitution of Thymine nucleotide with Adenine nucleotide in position 439 of GALNS gene was identified in the patient V.A.. Because of mutation in the protein a substitution of Tryptophan amino acid with Arginine amino acid in position 147 appears.

The same mutation was found in the patient A.A. in homozygous state: Thymine with Adenine substitution in position 439 with following mutation in the protein as substitution of Tryptophan amino acid with Arginine amino acid in position 147.

The GALNS variant c.439 T>A p. (Trp147Arg) causes an amino acid change from Trp to Arg at position 147. According to HGMD Professional 2019.1, this variant has previously been described as disease causing Mucopolysaccharidosis IVA by A. Rao and Bunge et al., 1997 (PMID: 9298823), Rivera-Colon et al., 2012 (PMID: 22940367 (PMID: 24411403). ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 197149) and likely pathogenic (clinical testing, Variation ID: 197149). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG. Pathogenic variants in GALNS gene are associated with autosomal recessive Mucopolysaccharidosis type IVA (Morquio A; OMIM®: 253000).

In patient V.G. we observed mutation: substitution of Adenine nucleotide with Guanine nucleotide in position 1283 (c.1283 A>G) of the gene in homozygous state, which leads to amino acid substitution of Glycine with Arginine in position 428 (p. Gln428Arg).

Given the pathologically reduced enzyme activity and clinical information provided, we consider this variant to be pathogenic (class 1) according to the recommendations of Centogene and ACMG.

Double heterozygous state – compound state – was revealed in V.G. patient. The first mutation is Guanine nucleotide substitution with Adenine nucleotide in position 157 (Gly53Arg), and the second one: Cytosine nucleotide substitution with Thymine nucleotide in position 553. In proteins there were the first change of Glycine amino acid to Arginine amino acid, and the second change of Proline amino acid to Serine amino acid in position 185.

The GALNS variant c.157 G>A p. (Gly53Arg) causes an amino acid change from Gly to Arg at position 53. According to HGMD Professional 2019.1, this variant has previously been described as disease causing Mucopolysaccharidosis IVA by Bunge et al., 1997 (PMID: 9298823), Rivera-Colon et al., 2012 (PMID: 2940367), ClinVar lists this variant as pathogenic (clinical testing, Variation ID: 281018). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG.

Identical female twins revealed double heterozygous state of two different mutations of GALNS gene: 1. Guanine nucleotide substitution with Thymine (c. 463 G-T, p. Gly133Cys), 2. Cytosine nucleotide change to Thymine nucleotide in position 1018 (c. 1018 G-T, p. Gly340Cys). There happened mutation c.463 G-T in the gene encoding site.

In 26-year-old patient Sh. S. (see **Tables 2** and **4**) we have found abnormal activity of arylsulfatase B enzyme, and in N-acetylgalactosamine-6-sulfate sulfatase enzyme mutation have been identified as substitution of Adenine nucleotide with Guanine nucleotide in position 443 (A>G) in homozygous state. There the following change of Histidine amino acid to Arginine amino acid in position 148 in enzyme exists.

The GALNS variant c.443 A>G p. (His148Arg) causes an amino acid change from His to Arg at position 148. According to HGMD Professional 2018.2, this variant has previously been described as disease causing Mucopolysaccharidosis IVA by Wang et al., 2010 (PMID: 20574428), Rivera-Colon et al., 2012 (PMID: 22940367). It is classified as pathogenic (class 1) according to the recommendations of Centogene and ACMG (please, see additional information below)

All GALNS gene mutations identified in our Republic have got nucleotide changes.

As to the frequency, during studies of 56 patients with MPS clinical diagnoses, 26 were identified with N-acetylgalactosamine-6-sulfate sulfatase enzyme

Table 4 – Genetic analysis results of Sh. S. patient

Gene	Variant coordinates	Zygosity	In silico Parameters	Allele frequencies	Type and classification
GALNS	Chr16 (GRCh37): g. 88904171 T>C NM_001323544.1: c. 443 A>G p. (His148Arg) Exon 6	Homo	PolyPhen: Probably damaging Align-GVGD: C0 SIFT: Deleterious Mutation Taster: Disease causing Conservation: nt high	Genom AD: - ESP: 1000 G: CentoMD: 0.000046	Missense Pathogenic (class 1)

deficiency and diagnosis MPS IV (Morquio disorder) confirmed with GALNS gene mutations, which correspond to MPS type IV. Frequency of MPS IV equals 46.4% of all patients diagnosed with MPS variants.

Reference analytics shows the following depict for different world populations.

M. Terzioglu et al., (2002) when screening 10 patients with MPS IVA of severe clinic in Turkey were lucky to identify 6 different mutations and two polymorphisms. Five mutations and one polymorphism are novel ones. In three cases authors observed one nucleotide deletion (389 delG, 929 delG, and 763 delT) and insertion of Thymine nucleotide (1232 - 1233 insT). Two other mutations were identified earlier: missense mutation (Q473X) and one nonsense mutation (P179S). In patients from Turkey there was already known E477 and one novel polymorphism W520 for patients with MPS IVA revealed.

When examining two kids from non-relative Turkish families, there were two different mutations in homozygous state: p. L390X in exon 11 and p. W141R in exon 4. The p. L390X mutation was associated with four novel polymorphisms in intron 2, intron 5 and intron 6 and one polymorphism previously described in exon 7 (L. Chkioua, et. al., 2014).

Study of 72 patients with MPS diagnosis from Tunisia was done. 12 patients out of 72 have got IVA type, which is the second in frequency list, right after MPS I type (16,7%) (L. Chkioua, 2015).

Yana Puckett et al. (2021) obtained information from 789 MPS patients during a 20-year period. Incidence of MPS in the US was found to be 0.98 per 100,000 live births. Prevalence was found to be 2.67 per 1 million. MPS I, II, and III had the highest incidence rate at birth (0.26/100,000) and prevalence rates of 0.70–0.71 per million. Birth incidences of MPS IV, VI, and VII were 0.14; 0.04 and 0.027 per 100,000 live births.

Agnieszka Jurecka et al. (2015) studied population of Poland and some European countries during timeline between 1970 and 2019. Frequency of MPS of all types for Poland population comprised 1.8 for 100,000 population. Total number of patients with five MPS types was 392. Frequency of MPS IVA and B types was on the fourth place among five types and equaled 0.13 for 100,000 live newborns. MPS incidence rate in Poland is lower than in other European countries such as the Netherlands, the Czech Republic or Germany. The similar frequency is seen in Sweden and Denmark.

Khan S.A., et al. (2017) carried out a study in Japan and Switzerland and made comparison with other countries. In Japan they revealed 467 patients with MPS during 1982 and 2009, their frequency was 1.53 for 100,000 live births. MPS type IV comprised 10% of all MPS diagnosed. In Switzerland all studies for 34 years (1975–2008) have shown 1.56 for 100,000 live births for all MPS types. In this group of patients MPS type IV was at the second place and comprised 24% of all patients with MPS's.

Summing up all our studies in Azerbaijan population we could conclude that 26 out of 56 patients were identified with GALNS gene mutation, corresponding to MPS IVA type (46.4%). Seven mutation types in homozygous, double heterozygous (compound) and heterozygous state were identified. All mutations have nucleotide substitution. Practical application of the results is being discussed.

Conclusion and perspectives of further research. Thus, for the first time populational study of mucopolysaccharidosis disorder by means of molecular-genetic modern complex has been carried out for the period of 2018–2022 in population of the Azerbaijan Republic.

Subject of the study was a group of 56 patients with MPS diagnosed, 26 of them were identified as patients with MPS type IVA disorder, that made 46.4% of all revealed patients with MPS's. Frequency of MPS IV overwhelmed over other types of disorder. That differ from previously published study results got for different groups in the world populations.

Molecular-genetic analysis allowed our identification of 7 GALNS gene mutation types GALNS: 553 C>T, 439 T>A, 1283 A>G, 157 G>A, 463 G-T, 1018 G-T and 443 A>G. All identified GALNS mutations in patients in our Republic are already known and described in books and articles.

We also study mutations of other MPS types.

Discussion of prophylaxis ways of MPS IVA type for the population of the Azerbaijan Republic is always in our agenda consulting families of reproductive age with genetic burden of MPS's.

References

1. Morquio L. Sur une forme de dystrophie osseuse familiale. *Archives de médecine des infants. Paris.* 1929;32:129-135. [French]
2. Tomatsu S, Fukuda S, Masue M, Sukegawa K, Fukao T, Yamagishi A, et al. Morquio disease: isolation, characterization and expression of full - length cDNA for human N-acetylgalactosamine-6-sulfate sulfatase. *Biochem Biophys Res Commun.* 1991 Dec 16;181(2):677-83. PMID: 1755850. doi: 10.1016/0006-291X(91)91244-7
3. Fukuda S, Tomatsu S, Masuno M, Ogawa T, Yamagishi A, Rezvi GM, et al. Mucopolysaccharidosis IVA: submicroscopic deletion of 16q24.3 and a novel R386C mutation of N-acetylgalactosamine-6-sulfate sulfatase gene in a classical Morquio disease. *Hum Mutat*. 1996;7(2):123-34. PMID: 8829629. doi: 10.1002/(SICI)1098-1004(1996)7:2<123::AID-HUMU6>3.0.CO;2-D

4. Hori T, Tomatsu S, Nakashima Y, Uchiyama A, Fukuda S, Sukegawa K, et al. Mucopolysaccharidosis type IVA: common double deletion in the N-acetylgalactosamine-6-sulfatase gene (GALNS). *Genomics*. 1995 Apr 10;26(3):535-42. PMID: 7607677. doi: 10.1016/0888-7543(95)80172-I
5. Tomatsu S, Fukuda S, Cooper A, Wraith James E, Ferreira P, Di Natale P, et al. Fourteen novel mucopolysaccharidosis IVA producing mutations in GALNS gene. *Hum Mutat*. 1997;10(5):368-375. PMID: 9375852. doi: 10.1002/(SICI)1098-1004(1997)10:5<368::AID-HUMU6>3.0.CO;2-B
6. Tomatsu S, Fukuda S, Masue M, Sukegawa K, Masuno M, Orii T. Mucopolysaccharidosis type IVA: characterization and chromosomal localization of N-acetylgalactosamine-6-sulfate sulfatase gene and genetic heterogeneity. *Am J Hum Genet*. 1992;51(suppl):A178.
7. Tomatsu S, Montano AM, Oikawa H, Dung VC, Hashimoto A, Oguma T, et al. Enzyme replacement therapy in newborn mucopolysaccharidosis IVA mice: early treatment rescues bone lesions? *Mol Genet Metab*. 2015 Feb;114(2):195-202. PMID: 24953405. PMCID: PMC4256128. doi: 10.1016/j.ymgme.2014.05.013
8. Masuno M, Tomatsu S, Nakashima Y, Hori T, Fukuda S, Masue M, et al. Mucopolysaccharidosis IVA: assignment of the human N-acetylgalactosamine-6-sulfate sulfatase (GALNS) gene to chromosome 16q24. *Genomics*. 1993 Jun;16(3):777-8. PMID: 8325655. doi: 10.1006/geno.1993.1266
9. Morís F, Gotor V. Selective aminoacylation of nucleosides through an enzymatic reaction with oxime aminoacyl esters. *Tetrahedron.* 1994;50(23):6927-6934. doi: 10.1016/S0040-4020(01)81345-4
10. Khan SA, Peracha H, Ballhausen D, Wiesbauer A, Rohrbach M, Gautschi M, et al. Epidemiology of mucopolysaccharidoses. *Mol Genet Metab*. 2017 July;121(3):227-240. PMID: 28595941. PMCID: PMC5653283. doi: 10.1016/j.ymgme.2017.05.016
11. Guest JD, Rao A, Olson L, Bunge MB, Bunge RP. The ability of human Schwann cell grafts to promote regeneration in the transected nude rat spinal cord. Exp Neurol. 1997 Dec;148(2):502-22. PMID: 9417829. doi: 10.1016/exnr.1997.6693
12. Sukegawa K, Nakamura H, Kato Z, Tomatsus S, Montano AM, Fukao T, et al. Biochemical and structural analysis of missense mutations in N-acetylgalactosamine-6-sulfate sulfatase causing mucopolysaccharidosis IVA phenotypes. *Hum Molec Genet.* 2000 May 22;9(9):1283-90. PMID: 10814710.doi: 10.1093/hmg/9.9.1283
13. Terzioglu M, Tokatli A, Coskun T, Emre S. Molecular analysis of Turkish MPS IVA (Morqiuo A) patients: identification of novel mutations in the N-acetylgalactosamine-6-sulfate sulfatase (GALNS) gene. *Hum Mutat.* 2002 Dec;20(6):477-8. PMID: 12442278. doi: 10.1002/humu.9088
14. Puckett Y, Mallorga-Hernández A, Montaño AM. Epidemiology of mucopolysaccharidoses (MPS) in United States: challenges and opportunities. *Orphanet J Rare Dis*. 2021 May 29;16(1):241. PMID: 34051828. PMCID: PMC8164808. doi: 10.1186/s13023-021-01880-8
15. Jurecka A, Ługowska A, Golda A, Czartoryska B, Tylki-Szymańska A. Prevalence rates of mucopolysaccharidoses in Poland. *J Appl Genet*. 2015 May;56(2):205-210. PMID: 25472774. PMCID: PMC4412687. doi: 10.1007/s13353-014-0262-5
16. Khan SA, Peracha H, Ballhausen D, Wiesbauer A, Rohrbach M, Gautschi M, et al. Epidemiology of mucopolysaccharidoses. *Mol Genet Metab*. 2017 May;121(3):227-240. PMID: 28595941. PMCID: PMC5653283. doi: 10.1016/j.ymgme.2017.05.016
17. Khedhiri S, Chkioua L, Elcioglu N, Laradi S, Miled A. Mutations and polymorphisms in N-acetylgalactosamine-6-sulfate sulfatase gene in Turkish Morquio A patients. *Pathol Biol (Paris).* 2014 Feb;62(1):38-40. PMID: 24411403. doi: 10.1016/j.patbio.2013.10.001
18. Chkioua L. Genetic Heterogeneity of 72 Patients with Mucopolysaccharidosis in Tunisia. *Int J New Technol Res*. 2015 Jul;1(3):01-06.
19. Rivera-Colón Y, Schutsky EK, Kita AZ, Garman SC. The structure of human GALNS reveals the molecular basis for mucopolysaccharidosis IVA. *J Mol Biol*. 2012 Nov 9;423(5):736-51. PMID: 22940367. PMCID: PMC3472114. doi: 10.1016/j.jmb.2012.08.020
20. Chuang CK, Lee CL, Tu RY, Lo YT, Sisca F, Chang YH, et al. Nationwide Newborn Screening Program for Mucopolysaccharidoses in Taiwan and an Update of the “Gold Standard” Criteria Required to Make a Confirmatory Diagnosis. *Diagnostics (Basel).* 2021 Aug 31;11(9):1583. PMID: 34573925. PMCD: PMC8465393. doi: 10.3390/diagnostics11091583
21. Yana E, Amboro FYP, Nurisman E, Hadiyati N. The Role Of The Polri In The Law Enforcement Of The Distribution Of Hate Speech In The City Of Batam, Indonesia. *Ganesha Law Rev*. 2021;3(1). doi: 10.23887/glr.v3i1.321
22. Pasieka A. *Hierarchy and pluralism: Living religious difference in Catholic Poland*. Palgrave Macmillan New York; 2015. 261 p. doi: 10.1057/9781137482860
23. Chan EYY, Chiu CP, Chan GKW. Medical and health risks associated with communicable diseases of Rohingya refugees in Bangladesh 2017. *Int J Infect Dis.* 2018 Mar;68:39-43. PMID: 29329951. doi: 10.1016/j.ijid.2018.01.001

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ГЕНЕТИКА МУКОПОЛІСАХАРИДОЗУ IV ТИПУ (ХВОРОБА МОРКІО) У ПАЦІЄНТІВ З АЗЕРБАЙДЖАНУ

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Резюме. *Мета.* Виявлення та генетичне дослідження хвороби мукополісахаридоз типу IVA серед хворих дітей з Азербайджанської Республіки.

Матеріал та методи. В Азербайджанській Республіці проведено генетичний аналіз хвороби мукополісахаридоз 56 пацієнтів віком від шести місяців до 28 років. Експериментальний матеріал зібрано у дитячих медичних закладах Республіки за період 2018-2022 років. Забір крові пацієнтів зроблено на DBS (Dry Blood Sample) картки. Для визначення активності ферментів був використаний метод флуориметрії. Для генетичних досліджень використовували комплекс сучасних молекулярних методів діагностики.

Результати. Всім пацієнтам був проведений ферментний аналіз для N-Acetyl-galactosamin-6-sulfatase (MPS IVA) та beta-galactosidase (МПС IVB). У 26 з 56 пацієнтів було виявлено порушення MPS IV в результаті проведеного скринінгу з використанням ферментів для всіх типів MPS для точного виявлення дефіциту ферменту N-ацетилгалактозамін-6-сульфатсульфатази, який є специфічним для MPS IVA. У восьми пацієнтів рівень ферменту був дуже низьким, і варіював від <0,1 (LOD) мкмоль/л/год до <0,3 (LOD) мкмоль/л/год, що характерно для гомозиготного або подвійного гетерозиготного стану, коли норма становить ≥ 2,0 моль/л/год. У 18 пацієнтів рівень активності ферменту N-ацетилгалактозамін-6-сульфатсульфатази був знижений майже вдвічі (<0,6 (LOD) мкмоль/л/год - <0,1 (LOD)), що говорить про гетерозиготний стан розладу. Ген GALNS (galactosamine (N-acetyl)-6-sulfatase) досліджували методом секвенування нового покоління. Більше 99% кодуючих ділянок цих генів було вивчено з глибиною читання не менше 50X. Середня глибина читання становить 1559 показань. До аналізу були включені сполуки екзон-інтрон (±10 п.н.). Класифікацію патогенності отриманих даних проводили згідно з «Посібником ACMG*». Ідентичні близнюки жіночої статі виявили подвійний гетерозиготний стан двох різних мутацій гена GALNS: 1. Заміна нуклеотиду гуаніну на тимін (c. 463 G-T, p. Gly133Cys), 2. Заміна нуклеотиду цитозину на нуклеотид тиміну в положенні 1018 (c. 1018 G-T, p. Gly340Cys). Відбулася мутація c.463 G-T у ділянці, що кодує ген. Усі мутації гена GALNS, виявлені в Азербайджанській Республіці, мають нуклеотидні зміни. У ході досліджень 56 пацієнтів з клінічними діагнозами MPS у 26 було виявлено дефіцит ферменту N-ацетилгалактозамін-6-сульфатсульфатази та діагноз MPS IV (розлад Моркіо), підтверджений мутаціями гена GALNS, які відповідають типу MPS IV. Частота MPS IV становить 46,4% від усіх пацієнтів із діагнозом варіантів MPS. Шляхом молекулярно-генетичного аналізу вдалося ідентифікувати 7 типів мутацій гена GALNS: 553C>T (p.Pro185Ser), 439T>A (p.Trp147Arg), 1283A>G (p.Gln428Arg), 157 G>A (p.Gly 53 Arg), 463 G-T (p.Gly133Cys), 1018 G-T (p.Gly340Cys) и 443A>G (p.His148Arg).

Висновки. У населення Азербайджанської Республіки виявлено хворобу Моркіо на підставі дефіциту ферменту N-Asetyl-galactosamin-6-sulfatase. Частота дефіциту ферменту серед обстежених становить 46,4%. За допомогою молекулярних методів діагностики ідентифіковано 7 типів мутацій гена GALNS.

Ключові слова: N-ацетилгалактозамін-6-сульфатсульфатаза, β-галактозидаза, фермент MPS IVA, ген, мукополісахаридоз, аутосомно-рецесивний тип успадкування.

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