

UDC 575.224.22:616-053

MOLECULAR CHARACTERISTICS OF SANFILIPPO SYNDROME IN PATIENTS FROM AZERBAIJAN REPUBLIC

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First time in Azerbaijan Republic we carried out medical-genetic study of four patients with Sanfilippo syndrome (Mucopolysaccharidosis Type III) and their family members.

Goal of this research was Sanfilippo syndrome genetic heterogeneity study. Experimental material was collected year 2018 through year 2023. Affected children from specialized medical centers in Baku city and Gyandzhe, as well as during field works in Republic's regions were examined. 56 patients among many were suspicious for lysosomal disease as Mucopolysaccharidosis. Four patients corresponded with Sanfilippo syndrome.

*Molecular analysis of genes SGSH (A), NAGLU (B), HGSNAT (C), GNS (D) with Sanger technique allowed identification of the following mutations: SGSH gene: NM_000199 c.7_16del, p.Cys3ProfsTer9 (A type); NAGLU gene: NM_c.389 GC, p.Arg130Pro (B type); two mutations of HGSNAT gene: NM_152418.2, c.852-1G-A in intron 9 and c.1345dup, p.Asp449Glyfs*21, in exon 13 (C type). All identified mutations were in homozygous state. In the ClinVar[®] list those mutations are registered as pathogenic. Consequently, parents of the patients were consanguinity of first cousin type. Patient with c.1345dup variant type was identified with heterozygous carriage type of beta-thalassemia (HBB gene: c.118C>T (p.Gln40Ter)).*

Keywords: Sanfilippo syndrome, Mucopolysaccharidosis, SGSH, NAGLU, HGSNAT, GNS, Sanger technique

Introduction

Mucopolysaccharidosis (MPS) is lysosomal storage disease and consists 25% of all human lysosomal storage diseases [1].

Mucopolysaccharidosis type III (Sanfilippo syndrome) - got its name after Doctor Silvestre Sanfilippo who described that disease in 1963 [2].

There are four subtypes of Sanfilippo syndrome and each of them occurs with enzyme deficiency.

Sanfilippo syndrome is autosome recessive inherited. It is rare encountered. Approximate frequency is 1:70 000-80 000 among alive newborns) [3,4].

There are several references describing clinical, biochemical and molecular mucopolysaccharidoses researches especially Sanfilippo syndrome

studies in patients from Azerbaijan Republic. Hence, this article is sequel in our studies [5,6].

Goal of our studies is Sanfilippo syndrome genetic heterogeneity identification using contemporary biochemical and molecular genetic diagnostics methods and techniques.

Materials and methods

Experimental material was collected starting year 2018 through year 2023. Work was conducted in specialized children medical centers in Baku city and Gyandzhe as well as during field studies in regions of the Republic.

Four out of 56 chosen patients suspicious with lysosomal disease as mucopolysaccharidosis corresponded to Sanfilippo syndrome (mucopolysaccharidosis type III).

Capillary or venous blood was sampled from patients to DBS (Dry Blood Spot) cards and 2 ml to tubes with anticoagulant EDTA or heparin.

To diagnose disease first of all four enzyme activity analyses were carried out for all four genetic types of the syndrome: heparan-N-sulfatase (Sanfilippo A), alpha-N-acetyl-D-glucosaminidase (Sanfilippo B), heparan acetyl-CoA-glucosaminid-N-acetyltransferase (Sanfilippo C), and N-acetyl glucosamine 6-sulfatase (Sanfilippo D). Enzyme activity was conducted with mass spectrometry.

To confirm results of enzyme analysis we carried out genetic test of the following genes: *SGSH (A)*, *NAGLU (B)*, *HGSNAT (C)* and *GNS (D)*. For DNA isolation "QIAamp DNA Blood mini kit" manufactured in Germany was used. Genetic test was carried out with the apparatus MiSeq Illumina®, manufactured by Illumina® (USA). Diagnostic panel included *SGSH*, *NAGLU*, *HGSNAT*, *GNS* genes. At the same time we conducted molecular analysis for HBB gene to test for presence of beta-thalassemia if any.

Genetic analysis was carried out with Sanger method. The following diagnostic kits were used: Lysosomal Storage Disease Kit, Celeomics®; Analytic platform - MiSeq Sequencing, Illumina®; Analysis platform - SEQ, GENOMIZE® (<http://seq.genomize.com/>), GRCh37(h19).

More than 99% gene coding sites were studied with reading depth of not less than 50X. Mean reading depth was 1559 indications.

Analysis included exon-intron linkage (± 10 n.p.). Pathogeny classification of the obtained results were conducted correspondently to "Guidelines of ACMG*".

Results and discussion

Four out of 56 with clinic manifestations of Mucopolisaccharidoses had clear exhibition of Sanfilippo syndrome. For each of syndrome four types there exists individual specific lysosomal enzyme.

All four patients showed zero activity of those specific enzymes. One case with full enzyme deficiency for enzymes heparan-N-sulfatase and alpha-N-acetyl-D-glucosaminidase each specific for types A and B were revealed. Two patients had full deficiency of enzyme heparan acetyl-CoA-glucosaminid-N-acetyl transferase specific for C type.

In all cases complete deficit of enzyme (0.0 (LOD) $\mu\text{mol/L/h}$) was specifically attributed to gene homozygous or double heterozygous state (compound) when norm was ≥ 2.0 mol/L/h.

Enzyme analysis of thirteen family members - eight parents and five sibs showed following condition. All parents revealed enzyme deficiency corresponded to heterozygous carriers. And four of five sibs had enzyme deficit. Levels varied between 0.9 mol/L/h and 1.2 mol/L/h. Results of enzyme analysis are presented in Table 1.

Table 1

Sanfilippo syndrome patients' enzyme analyses results

Patient	Enzyme	Syndrome type	Activity	Zygosity	Technique
B.M.	Heparan-N-sulfatase	Sanfilippo A	0 $\mu\text{mol/L/h}$	Homozygote or compound	Mass spectrometry
B.J.	Alpha-N-acetyl-D glucosaminidase	Sanfilippo B	0 $\mu\text{mol/L/h}$	Homozygote or compound	Mass spectrometry
G.I.	Heparan acetyl-CoA-glucosaminid-N-acetyltransferase	Sanfilippo C	0 $\mu\text{mol/L/h}$	Homozygote or compound	Mass spectrometry
T.M.	Heparan acetyl-CoA-glucosaminid-N-acetyltransferase	Sanfilippo C	0 $\mu\text{mol/L/h}$	Homozygote or compound	Mass spectrometry

For genetic diagnostics there was necessary to conduct DNA molecular analysis.

Patient B.M. (female) 2017 born, had got zero activity of heparan-N-sulfatase (0.0 (LOD) $\mu\text{mol/L/h}$). We managed to identify c.7_16del mutation in SGSH gene: (NM_000199.5) in homozygous state. That mutation was also identified in parents and two siblings. Parents of the index patient had consanguineous marriage (first cousin kindred). On the protein level the following change took place: substitution of amino acid Cysteine with amino acid Proline in the position 3 that completed with terminating codon 9 (p.Cys3ProfsTer9).

Genetic analysis result of index patient with Sanfilippo A syndrome is presented in Fig. 1.

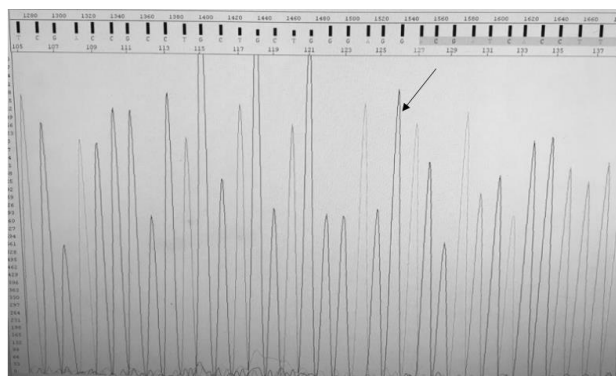


Fig. 1. Patient B.M.'s genetic analysis of SGSH gene: NM_000199.5. c.7_16del

SGSH gene of heparan-N-sulfatase was identified in 1995. It located in Chromosome 17 in 17q25.3 locus. Sanfilippo A syndrome is most widely spread subtype. That syndrome comprises 60% of all subtypes of disease [5,8,13]. Epidemiological researches in British Columbia since 1952 through 1986 revealed 4 cases of Sanfilippo A syndrome, that was equal to 1: 324 617 alive newborns. Frequency for Sanfilippo A syndrome in Netherlands was estimated as **1.16-0.88: 100 000 alive newborns** [7].

The second index patient B.J. (male) 2014 born revealed Sanfilippo B syndrome with mutation - substitution of nucleotide Guanine with nucleotide Cytosine in position 389 by means of genetic analysis of NAGLU gene (Gene ID: 4669). Consequence of that mutation was substitution of amino acid Arginine with amino acid Proline in position 130 (c.389G>C, p.Arg130Pro). Frequency of

Sanfilippo B syndrome in population of Netherlands and Australia equaled to 34.2% and 45.5% of all subtypes, relatively (8).

Genetic analysis of HGSNAT gene in two patients with full activity deficit of heparan acetyl-CoA-glucosaminid-N-acetyltransferase enzyme revealed two different mutations. Index patient G.I. (male) 2006 born, identified mutation c.852-1G>A in intron 9. Mutation c.852-1G>A of HGSNAT gene destroys highly conserved splice-site of acceptor of exon 10. According to HGMD Professional 2019.1., this mutation was earlier described as disorder causing MPS IIIC (Sanfilippo C syndrome). Parents of the patients were close relatives - cousins.

The second index patient T.M. (male) 2006 born, with heparan acetyl-CoA-glucosaminid-N-acetyltransferase zero enzyme activity identified the following mutation: c.1345dup. That occurred due to mutation in exon 13 of HGSNAT where reading frame shift was created starting codon 449 (Asp449Glyfs*21). Substitution of amino acid on the level of synthesized protein took place, and effect of mutation was substitution of amino acid Arginine with amino acid Glycine in position 449 (Asp449Glyfs*21). New reading frame completed with development of stop-codon 20, according to HGMD Professional 2019.1.

This mutation was described earlier as a disorder causing MPS IIIC. In ClinVar® list this mutation was registered as pathogenic. Clinical testing identified variant under ID: 1231. It was classified as pathogenic (class 1) in accordance with Recommendations of Centogene® and ACMG®.

Result of genetic analysis of index patient T.M. with Sanfilippo C syndrome presented in Fig. 2.

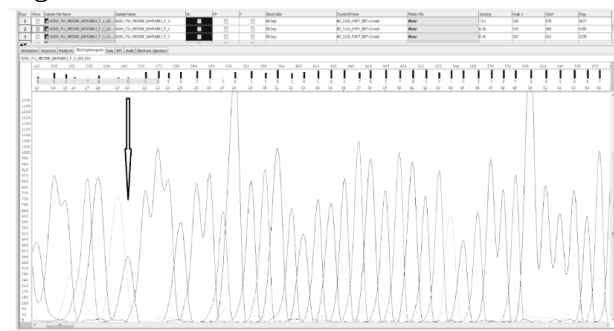


Fig. 2. Patient T.M.'s genetic analysis of HGSNAT gene (c.852-1G>A)

Table 2

Sanfilippo syndrome subtypes, their location in chromosomes, phenotype, gene name, locus number and enzyme name

Subtype	Location	Phenotype	Gene	Locus	Enzyme
MPS IIIA	17q25.3	252900	<i>SGSH</i>	605270	Heparan-N-sulfatase
MPS IIIB	17q21.2	252920	<i>NAGLU</i>	609701	Alpha-N-acetyl-D-glucosaminidase
MPS IIIC	8p11.21	252930	<i>HGSNAT</i>	610453	Heparan acetyl-CoA-glucosamenid-N-acetyltransferase

In ClinVar[®] list this mutation was registered as pathogenic (clinical testing, variant ID: 556501). It was classified as pathogenic (class 1) in accordance with Recommendation of Centogene[®] and ACMG[®].

Epidemiologic studies in Western Australia since 1969 through 1998 years identified 11 cases with MPS III (1:58 000), 5 cases with MPS III A and MPS IIIB, and one patient was found out as a case with MPS IIIC [9].

Khan et al., 2017 studying 467 patients in Japan and Switzerland during 1982-2009 years diagnosed cases with all MPS types. Frequency was evaluated as 1.53: 100 000 live newborns. MPS III was 16% of all affected with MPS (0.84:100 000) [10,11].

Prevalence of Sanfilippo syndrome in different countries of the world varied greatly: around 1: 280 000 in newborns in Northern Ireland up to 1: 66 000 in Australia and 1: 50 000 in Netherlands. Type A was most spread in North-West Europe, type B - in South-East Europe, types C and D are rarely encountered. [12,13].

In Table 2 we presented all subtypes of Sanfilippo syndrome. Index patient T.M. with *SGSH* gene deletion: NM_000199.5: c.7_16del showed heterozygous carriage of beta-thalassemia. *HBB* gene analysis found out substitution of nucleotide Cytosine with nucleotide Thymine in position 118 and, the consequent protein change of amino acid Glycine with amino acid Tyrosine (Gln40Ter).

Thus, out of four Sanfilippo types we found out three types: A, B, and C. They were studied on biochemical level with enzyme activity indication as well as *SGSH*, *NAGLU* u *HGSNAT* gene mutations were identified using contemporary diagnostic molecular techniques. *Two identified mutations were qualified as mis-*

*sense mutations and the rest two: one - deletion and one - duplication. Unique compound of Sanfilippo syndrome (*SGSH*: c.7_16del.) and beta-thalassemia (*HBB*: c.118C>T) in heterozygous state was discovered.*

Conclusions

- For the first time medical-genetic studies of affected children in Azerbaijan Republic were conducted to identify and study genetic heterogeneity of Sanfilippo syndrome.

- Enzyme analysis of all four subtypes of Sanfilippo syndrome identified full deficiency of three enzymes: heparan-N-sulfatase, alpha-N-acetyl-D-glucosaminidase, heparan acetyl-CoA-glucosamenid-N-acetyltransferase correspondent to A, B and C subtypes. Zero activity of enzymes predicted homozygous state of disease.

- One patient with zero activity of heparan-N-sulfatase (0.0 (LOD) $\mu\text{mol/L/h}$) corresponding to Sanfilippo A syndrome genetic test on *SGSH* gene level deletion in the gene: NM_000199.5: c.7_16del, p.Cys3ProfsTer9 was identified in homozygous state.

- Genetic analysis of *NAGLU* gene (Gene ID: 4669) in patient with full enzyme deficiency of alpha-N-acetyl-D-glucosaminidase identified nucleotide Guanine substitution with nucleotide Cytosine in position 389 (G>C).

- Two patients with zero enzyme activity of heparan acetyl-CoA-glucosamenid-N-acetyltransferase underwent genetic analysis of *HGSNAT* gene identified two different mutations: c.852-1G-A mutation in intron 9 that destroyed highly conserved splice cite of exon10 acceptor, according to HGMD Professional 2019.1. And in the second patient, a mutation (c.1345dup.) was identified and, it caused in

consequence creates reading frame shift in exon 13 starting with codon 449 (Asp449Glyfs*21).

- Unique compound variant of two diseases were identified: Sanfilippo syndrome (SGSH: c.7_16del) in homozygous state and beta-thalassemia (HBB: c.118C>T) in heterozygous state.

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AZƏRBAYCAN RESPUBLİKASINDA SANFİLİPPO SİNDROMLU PASİENTLƏRİN MOLEKULAR-GENETİK XARAKTERİSTİKASI

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İlk dəfə olaraq Azərbaycan Respublikasında Sanfilippo sindromlu (mukopolisaxaridoz III tip) dörd pasientin molekulyar-genetik xarakteristikası tədqiq edilmişdir. Eksperimental material respublika rayonlarından, o cümlədən Bakı və Gəncə şəhərlərinin uşaq xəstəxanalarından, 2018-2023-cü illər ərzində toplanmışdır.

SGSH (A), NAGLU (B), HGSNAT (C), GNS (D) genlərinin Senqer metodu ilə tədqiqi Sanfilippo sindromunun

aşağıdakı mutasiyaları identifikasiya edilmişdir: gen SGSH: NM_000199 c.7_16del,p.Cys3ProfsTer9 (A); gen NAGLU: NM_ c.389 GC, p.Arg130Pro (B); HGSNAT geninin iki mutasiyası: NM_152418.2, 9 sayılı intronda c.852-1G-A və 13 sayılı ekzonda c.1345dup, p.Asp449Glyfs*21 (C). İdentifikasiya edilmiş mutasiyalar homoziqot halda olmuşlar. ClinVar® siyahısında bu mutasiyalar patogen qeyd edilmişdir. c.1345dup mutasilalı pasientdə beta-talassemiyanın HBB geninin c.118C>T (p.Gln40Ter) mutasiyasının heteroziqot daşıyıcılığı identifikasiya edilmişdir.

Açar sözlər: mukopolisaxaridoz III tip, Sanfilippo sindromu, SGSH, NAGLU, HGSNAT, GNS, Senqer metodu

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА СИНДРОМА САНФИЛИППО У ПАЦИЕНТОВ ИЗ АЗЕРБАЙДЖАНСКОЙ РЕСПУБЛИКИ

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Впервые в Азербайджанской Республике с целью изучения молекулярно-генетической характеристики исследовано четыре пациента с синдромом Санфилиппо (мукополисахаридоз III типа). Экспериментальный материал был собран в регионах Республики, включая детские медицинские учреждения в Баку и Гяндже, с 2018 г. до 2023 г. включительно.

Молекулярный анализ генов SGSH (A), NAGLU (B), HGSNAT (C), GNS (D) методом Сэнгера позволил идентифицировать следующие мутации: ген SGSH: NM_000199 c.7_16del, p.Cys3ProfsTer9 (A); ген NAGLU: NM_ c.389 GC, p.Arg130Pro (B); две мутации гена HGSNAT: NM_152418.2, c.852-1G-A в интроне 9 и c.1345dup, p.Asp449Glyfs*21, в экзоне 13 (C). Все идентифицированные мутации были в гомозиготном состоянии. В списке ClinVar® эти мутации отмечены как патогенные. У пациента с мутацией c.1345dup, идентифицировали также и гетерозиготное носительство бета-талассемии (ген HBB^c.118C>T (p.Gln40Ter)).

Ключевые слова: мукополисахаридоз III типа, синдром Санфилиппо, SGSH, NAGLU, HGSNAT, GNS, метод Сэнгера