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Sanfilippo A Syndrome Genetic Studies in the Patient from Azerbaijan Republic

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Abstract

First time in the patient from Azerbaijan Republic, genetics of Mucopolysaccharidosis Type III - Sanfilippo A syndrome was identified and studied. In order to study Sanfilippo A genetics, authors used complex of modern molecular-genetic methods and techniques. During patient's medical-genetic consultation with doctor pediatrician and doctor geneticist in the Central Hospital of Gyanja City, a patient, originated from Lachin region of the Republic, was identified as an affected one relied on the clinical manifestations. Parents had a consanguineous marriage first cousins. Clinical studies showed cognitive changes, speech disorder, aggressiveness, hyperactivity, autistic signs, troubles in motions, hepatomegaly, failure in gaining weight, and physical development lag. For diagnostics capillary and venous blood samples were used. Blood from the patient was sampled into 2 ml-tubes with anticoagulant and absorbed to DBS (Dry Blood Spot) cards. Urine analysis was carried out with thin-layer chromatography. Enzyme activity was valued with mass spectrometry technique, and the DNA level analysis for SGSH gene was conducted with NGS technique. Urine analysis results allowed us to diagnose provisionally Sanfilippo syndrome MPS III based on increased values of heparan sulfate and keratan sulfate. Specification of Sanfilippo syndrome type (A, B, C or D) was conducted with activity evaluation of all four lysosomal enzymes: N-sulfoglucosamine sulfohydrolase (for TypeA), alpha-N-acetyl-D-glucosea-minidase (for Type B), heparan acetyl-CoA-glucoseaminide-N-acetyltransferase (for Type C), N-acetylglucoseamine-6-sulfatase (for Type D). We have got N-sulfoglucosamine sulfohydrolase 0 activity specific for Sanfilippo A syndrome. Molecular analysis identified mutation c.7_16del, p.Cys3ProfsTer8 of SGSH gene: (NM_000199.5) in homozygous state. Taking into account reproductive age of parents, fetus prenatal diagnostics is being planned for the next pregnancies.

Key words: Sanfilippo A syndrome, Mucopolysaccharidosis Type III, N-sulfoglucosamine sulfohydrolase, enzyme, SGSH gene, NGS technique, sequencing

Introduction

Sanfilippo syndrome (Mucopolysaccharidosis Type III) is named after Doctor Sylvester Sanfilippo who first described this disorder in 1963. Sanfilippo syndrome is a hereditary lysosomal storage disorder, genetically heterogeneous, conditioned with heparin sulfate (HS) storage and specified with progressing mental retardation, and moderate skeletal alterations (Aronovich et.al., 2000; Cleary et.al., 1993; Cross et.al., 2014; Esposito et.al., 2000).

Four subtypes of Sanfilippo Syndrome were identified, each of them arise with enzyme deficiencies: N-sulfoglucoseamine sulfohydrolase (Sanfilippo A), Alpha-N-acetyl-D-glucoseaminidase (Sanfilippo B), Heparan acetyl-CoA-glucoseaminide-N-acetyltransferase (Sanfilippo C), N-acetylglucoseamine-6-sulfatase (Sanfilippo D). Those enzymes' genes are located on 8, 12 and 17 chromosomes. Each enzyme deficiency leads to heparan sulfate (HS) (Esposito et.al., 2000). Every type of this disease is inherited as autosome-recessive, and parents of the index patient are heterozygotes on pathologic gene (Aronovich et.al., 2000; Bhattacharyya et.al., 2001; Valstar et.al., 2010; Xiong et.al., 2015).

Sanfilippo A type causing N-sulfoglucoseamine sulfohydrolase gene (SGSH: 605270) is located on the long shoulder of chromosome 17 (17q25) and modifies synthesis of the same named enzyme N-sulfoglucoseamine sulfohydrolase (Esposito et.al., 2000).

Sanfilippo syndrome takes the third place according to frequency among all nowadays known mucopolysaccharidoses (Khan et.al., 2017; Valstar et.al. 2008).

In British Columbia province, Canada only four out of 325617 examined newborns had got Sanfilippo syndrome (0.0012%). N-sulfoglucoseamine sulfohydrolase (Lowry et.al., 1990). In West Australia five newborns out of 58000 manifested and identified Sanfilippo A type syndrome, where frequency was counted as 0.0086% (Nelson et.al., 2003). In Netherlands frequency of the disease was 0.88-1.15:100000 among alive newborns (Poorthuis et.al., 1999).

It should be noticed that the problem in diagnostics and treatment of lysosomal storage diseases exists, and particularly of mucopolysaccharidoses. In consequence, goal of our studies is to study molecular basics of Sanfilippo syndrome using modern molecular-genetic methods and techniques of diagnostics, taking into account unexplored the disease genetics for the Azerbaijan Republic population.

Materials and methods

Index patient is a 4-year-old girl examined by doctor pediatrician and doctor geneticist in Central Hospital in Gyanja city in 2021. As to clinical manifestations the disease was provisionally diagnosed as lysosomal disease Mucopolysaccharidosis and specifically Sanfilippo syndrome. For this diagnostics urine, and 2 ml capillary and venous blood samples were collected on DBS (Dry Blood Spot) cards and tubes with anticoagulant. Urine analysis was carried out with thin-layer chromatography technique.

To identify disease type the following enzymes were used: N-sulfoglucoseamine sulfohydrolase (Sanfilippo A), Alpha-N-acetyl-D-glucoseaminidase (Sanfilippo B), Heparan acetyl-CoA-glucoseaminide-N-acetyltransferase (Sanfilippo C) and N-acetylglucoseamine-6-sulfatase (Sanfilippo D). Enzymes activity evaluation was conducted with mass-spectrometry technique.

DNA obtained from patient's peripheral blood sample was studied with NGS (New Generation Sequencing) technique. «A custom double stranded DNA capture bait pool was used to selectively enrich the coding regions, 10 bp of flanking intronic sequences, and known relevant variants beyond the coding regions, based on HGMD® and CentoMD® for the 166 panel genes. Libraries are generated with Illumina compatible adaptors, and sequenced on an Illumina platform to obtain ≥ 50x coverage depth for >99.5% of the targeted bases. Mean depth of reading consists of 1559 indications. All potential disease-causing variants, including the ones reported in HGMD®, in ClinVar and in CentoMD® are considered. The investigation for relevant variants is focused on coding exons and flanking +/-10 intronic bases. All potential modes of inheritance patterns are considered. Centogene® has established stringent quality criteria and validation processes for variants detected by NGS. Pathogeny classification of the obtained results was considered according to «Guidelines of ACMG*». At the same time sequencing of SGSH gene was carried out.

Results and discussions

During genetic consultation, doctor pediatrician and doctor geneticist examined genetically burdened patients in Central Hospital of Gyanja city, and identified a four-year-old patient with clinical manifestations of lysosomal storage disease which is Mucopolysaccharidosis Type III (MPS III).

When examining clinically, doctors observed cognitive change, cognitive changes, speech disorder, aggressiveness, hyperactivity, autistic signs, troubles in motions, hepatomegaly, failure in gaining weight, and physical development lag. Patient's photo is presented in **Figure 1**.

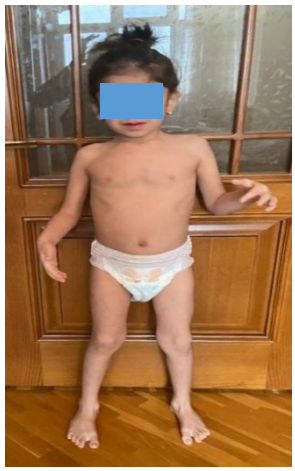

Figure 1. Patient's photo suspicious with MPS III (Sanfilippo syndrome)

Patient was born from the consanguineous marriage (parent's mothers are sisters). Parents were originated from Lachin region of the Republic.

First of all urine analysis was carried out for lysosomal storage disease - Mucopolysaccharodosis, and that was conducted with thin-layer chromatography technique. Increase of glucoseaminoglycans (GAG) - 52.12 mg/µmol when norm must be 7.60-14.40 mg/µmol because of heparan sulfate (HS) increase and keratan sulphate (KS) for Sanfilippo syndrome (MPS III).

MPS III is heterogeneous and has four subtypes. Consequently, for diagnostics and identification of Sanfilippo syndrome, the following enzymes activity evaluation was used: N-sulfoglucoseamine sulfohydrolase (Sanfilippo A), Alpha-N-acetyl-D-glucoseaminidase (Sanfilippo B), Heparan acetyl-CoA-glucoseaminide-N-acetyltransferase (Sanfilippo C) and N-acetylglucoseamine-6-sulfatase (Sanfilippo D). Enzyme analysis was conducted in dried blood samples from DBS (Dry Blood Spot) cards.

Enzyme analysis results showed 0-activity only for one enzyme - N-sulfoglucoseamine sulfohydrolase - that corresponds with Sanfilippo A syndrome. The patient was suspicious of Sanfilippo A syndrome, and her family members' enzyme analyses results are presented in Table 1.

Table 1. Enzyme analyses results of Sanfilippo A syndrome patient family members.

Patient	Result	Reference	Zygosity	Inter-pretation	Method
Index patient	0 (LOD) μmol/L/h LOD = limit of quantification	≥ 5,0 μmol/L/h	Homozygote	Pathologic, class 1	mass spectrometry
Mother	1,81 (LOD) μmol/L/h LOD = limit of detection	≥ 5,0 μmol/L/h	Heterozygote	Pathologic, class 1	mass spectrometry
Father	2,0 (LOD) μmol/L/h LOD = limit of detection	≥ 5,0 μmol/L/h	Heterozygote	Pathologic, class 1	mass spectrometry

As it is seen from the Table 1, patient showed 0-activity of N-sulfoglucoseamine sulfohydrolase enzyme that corresponds with homozygous state of enzyme deficit. To genetically confirm the diagnosis Sanfilippo A syndrome we carried out SGSH gene genetic analysis. This analysis was conducted with two methods: NGS technique and sequencing method. Results of SGSH gene sequencing are presented in Figure2.

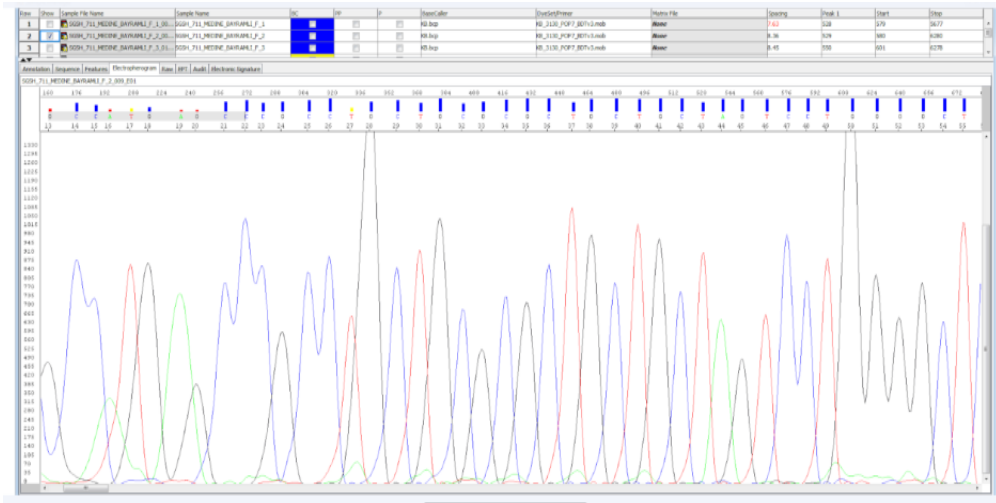

Figure 2. SGSH gene sequencing results

We coped to identify SGSH gene mutation c.7_16del гена SGSH: (NM_000199.5) for patient with 0-activity of N-sulfoglucoseamine sulfohydrolase enzyme. Here

deletion of 10 bp from 7 to 16 nucleotides is observed. As a result a substitution of amino acid Cysteine with amino acid Proline in codon 3 exists on the level of protein (enzyme). Mutation leads to formation terminating codon 8. The said mutation is pathogenic class 1 (see Table 2).

Table 2. SGSH and HGSNA genes genetic analyses results

MPS III	Gene	Gene mutation	Protein
Sanfilippo A syndrome	SGSH: NM_000199.5	c.7_16del	p.Cys3ProfsTer8

Patient manifests homozygous state of the mutation. Both parents had heterozygous carriage of this mutation.

Sanfilippo A syndrome is the mostly spread subtype. Course of the disease in this form is the severest with early onset and speediest progressing symptoms and short life expectancy (Bhattacharyya et.al., 2001).

Frequency for MPS in Japan valued 1.43 for 100000 among live newborns. Sanfilippo syndrome comprised only 16% out of all MPS types. In Switzerland MPS counted 1.56 for 100000 newborns. And MPS III considered 24% for all MPS types, Average frequency of Sanfilippo A syndrome here comprised 60% among all MPS disease types. Epidemiologic studies in British Columbia province of Canada identified 4 cases of Sanfilippo A syndrome between years 1952 and 1986, and that was counted as 1: 325,617 living newborns. (Esposito et.al., 2000; Khan et.al., 2017; Lowry et.al., 1990; Nelson et.al., 2003).

Thus, in patient suspicious with lysosomal disease Mucopolysaccharidosis Type III - Sanfilippo syndrome - genetics of the disease is studied at the level of glycosaminoglycan in urine and the level of enzyme and SGSH gene. SGSH gene mutation was identified: c.7_16del (NM_000199.5) in homozygous state. Taking into account parents' reproductive age, fetus prenatal diagnostics is recommended during the course of next pregnancies.

Conclusion

During genetic consultation, doctor pediatrician and doctor geneticist examined and identified patient with clinical manifestations of mucopolysaccharidosis - lysosomal storage disease.

2. On the basis of results of thin-layer chromatography urine analysis that showed increased values of heparan sulfate and keratan sulfate we were able to identify provisional diagnosis as Sanfilippo syndrome (MPS III).

3. Patient's blood sample was analyzed with all four enzymes for identification certain type of Sanfilippo syndrome (MPS III). As a result we got 0-activity for N-sulfoglucosamine sulfohydrolase enzyme that confirms Sanfilippo A syndrome in the patient.

4. Molecular analysis identified SGSH gene mutation c.7_16del, p.Cys3ProfsTer8: (NM_000199.5) in homozygous state.

5. Taking into account reproductive age of parents, fetus prenatal diagnostics is being planned for the next pregnancies.

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