Genotype to Phenotype: Identification of Mucopolysaccharidosis Type IIIB (Sanfilippo's B) Case Using Whole Exome Sequencing

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Abstract

Mucopolysaccharidosis type IIIB (Sanfilippo's B; OMIM no.: 252920) is a lysosomal storage disorder caused by defective degradation of heparan sulfate. The enzyme that has decreased function in this disease is α-N acetylglucosaminidase. This enzyme is encoded by the *NAGLU* gene. A 9-year-old male patient was referred to us with speech disability, developmental delay, hepatomegaly, mild learning disability, and otitis media with effusion complaints. Whole exome sequencing (WES) was performed because of consanguinity between the parents of the patient and the lack of specific prediagnosis. As a result of the patient's WES analysis, a homozygous mutation was detected in the *NAGLU* gene. The leukocyte enzyme activity was then evaluated to confirm the diagnosis. Alpha-N acetylglucosaminidase deficiency was found. Alpha-N acetylglucosaminidase activity was 0.2 nmol/mLh. WES is a successful diagnostic method in the diagnosis of the mild clinical diseases with recessive inheritance. In addition, our case is a good example of genotype to phenotype diagnosis. Because in storage diseases, the diagnosis is made by leukocyte enzyme analysis first, and then the result is confirmed by gene analysis. The opposite situation occurred in our case.

Keywords
- mucopolysaccharidosis III
- Sanfilippo's syndrome B
- consanguineous marriage
- whole exome sequencing

Introduction

The characteristics of the group of lysosomal storage disease that have approximately 40 diseases so far are as follows: skeletal abnormalities, organ dysfunction, and neuronal involvement. The etiopathology for this group of diseases is the accumulation of substances such as glycosaminoglycans, glycosylated proteins, or membrane lipids.[1] There are 10 lysosomal storage diseases caused by the lack of enzymes required for the destruction of glycosaminoglycans (GAG). This group of diseases is mucopolysaccharidosis (MPS).[2]

MPS III or Sanfilippo's syndrome is characterized by the dysfunction of four lysosomal enzymes that cause degradation of heparan sulfate. These four lysosomal enzymes and the types of MPS III are as follows; heparan N-sulfatase (MPS IIIA, OMIM no.: 252900), α-N acetylglucosaminidase (NAGLU; MPS IIIB, OMIM no.: 252920), acetyl-CoA: α-glucosaminide acetyltransferase (MPS IIIC, OMIM no.: 252930), and N acetylglucosamine 6-sulfatase (MPS IIID, OMIM no.: 252940).[3] All four of these diseases are inherited in an autosomal recessive manner. The *NAGLU* gene contains six exons and spans 8.3 kB.

It is difficult to distinguish between MPS III types based on clinical and symptoms. The clinical presentation of the disease is divided into three phases. In the first months of life, a symptom-free period is seen before the clinical manifestations of the disease begin to appear. The first phase of

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the disease starts with the slowing of mental development between 1 and 4 years. The second phase of the disease begins to be seen with severe behavioral problems and progressive intellectual decline at approximately 3 to 4 years of age. In the last phase, onset of severe dementia, the slow disappearance of behavioral problems, and the regression of all motor functions begin.[4] The expected symptoms in this disease are as follows: hyperactivity, aggressive behavior, delayed development (particularly in speech), sleep disturbances, coarse face, hirsutism, and diarrhea.[5]

The aim of this study was to present a broad spectrum of MPS III disease.

Case Report

A 9-year-old male patient was referred to us with speech disability, developmental delay, hepatomegaly, mild learning disability, ptosis of the left eye, and otitis media with effusion complaints. In the anamnesis information of the patient, it was learned that the prenatal period was normal. The birth information of the patient included 3,000-g weight with spontaneous vaginal delivery at term. The patient did not have a history of hypoxic birth and the Apgar score at 5 minutes was 8 to 9. When the motor developmental stages of the patient were examined, mild retardation was detected (head and neck control at month 4, sitting with supporting at month 6, sitting unsupporting at month 8, and walking at month 14). When the patient's previous surgeries are questioned, it was revealed that he had two surgeries. One for adenoid hypertrophy operation and the other for tympanostomy tubes for otitis media. The patient has frequent upper respiratory tract infections.

The pedigree of the patient revealed that the parents were consanguine (1st degree cousins). In the family, there was no other case similar to our patient.

Physical examination revealed that the height, weight, and head circumference of the patient were normal according to his age. At the dysmorphic examination square face, malar flatting, underdeveloped nasolabial fold, broad jaw, broad chin, epicanthus, hypertelorism, downslanted palpebral fissure, telecanthus, prominent anithelix stem, serpenginous antihelix stem, everted antitragus, low-set ear, protruding ear, prominent crus helix, uplifted lobe, short columella, anteverted nares, wide nasal bridge, and thick lower lip vermillion were found. The patient had mild intellectual disability. There was also a mild delay in speech of the patient, as recorded. Abdominal examination revealed hepatomegaly.

No pathological value was found in the biochemical laboratory examinations of the patient. Abdominal ultrasonographic examination of the patient was measured as enlargement of the liver. At the same time, expansion in the spleen was also observed. Echocardiographic examination shown asymmetric hypertrophy in the interventricular septum and mitral valve prolapse. Magnetic resonance imaging (MRI) showed mild thinning of the corpus callosum. No specific prediagnosis could be made for the patient. Because of the consanguinity between parents, whole exome sequencing (WES) was planned. As a result of WES, homozygous c.1694G > A (p.Arg565Gln) (p.R565Q) missense mutation was detected in the *NAGLU* gene. This mutation was then confirmed by Sanger's sequencing. This mutation causes MPS type IIIB (Sanfilippo's B). The DANN (Deleterious Annotation of genetic variants using Neural Networks) score of this mutation is 0.9995. At the same time the total allele frequency of the mutation was found to be 0.00003228. The leukocyte enzyme activity was then evaluated to confirm the diagnosis. Alpha-N acetylglucosaminidase deficiency was found; alpha-N acetylglucosaminidase of 0.2 nmol/mLh. Heparan sulfate excretion in urine was increased. Urine glycosaminoglycan was nine times higher than the reference range in the patient's age group (98.60 mg/L). MPS type IIIB (Sanfilippo's B) was diagnosed. To evaluate the dysostosis multiplex, spinal X-ray was performed. But no dysostosis multiplex sign was found.

Discussion

The clinical findings of MPS type III are in a broad spectrum from mild to severe.[6,7] The previously reported MPS type IIIB (Sanfilippo's B) patients have much more severe findings compared with our patient. In their study, Hettiarachchi et al reported a case with hypotonia, progressive loss of head control, and global developmental delay. The patient also had a homozygous mutation in the *NAGLU* gene.[8] In another MPS type IIIB case, presented by Rezayi et al, A 9-year-old boy had speech regression, seizure, and ataxia. He was developmentally normal until 4 years. Also, he has a mutation in the *NAGLU* gene. WES analysis performed and found mutation in *NAGLU* gene.[9] In our case, with speech disability, developmental delay, hepatomegaly, and mild learning disability findings, a homozygous mutation was detected in the *NAGLU* gene. The clinical symptoms of our patient are not severe. The phenotype of the disease is very diverse, suggesting that this diversity is due to the mutation point in the gene. Clinical synopsis findings of MPS type IIIB (Sanfilippo's B) in OMIM and their comparison with our case are summarized in ►**Table 1**. As it is seen here, our case has mild findings.

The presence of mild symptoms in the diagnosis stage of the patient could not direct us to a specific disease group. We thought that WES would be the best test to choose because of the fact that there was a consanguineous marriage between parents. And indeed, the patient's disease was diagnosed with WES. Our case is an example where WES is the best method, as there is presence of consanguineous marriage and also a specific prediagnosis cannot be considered. At the same time, this case is an example for diagnosis from genotype to phenotype.

Jain et al report a mild phenotype similar to our patient. Their patients had hepatomegaly, delayed motor development, and behavioral problems.[10]

With the development of technology, diagnosis has become easier in genetic diseases. Especially after the widespread WES test, diagnosis of diseases with recessive inheritance has accelerated. However, it should be kept in mind that if a good anamnesis is taken and the patient is evaluated in detail, the mutation can be evaluated well. Therefore, no matter how far the technology progresses, the importance of clinical examination and good anamnesis will never decrease.

Table 1 Comparison of the patient's findings with the clinical synopsis at OMIM

Clinical synopsis at OMIM	Presence of findings in our patient
Inheritance	
Autosomal recessive	+
Head and neck	
Coarse facies, mild	+
Hearing loss	+
Synophrys	+
Cardiovascular	
Asymmetric septal hypertrophy	+
Cardiomegaly	−
Abdomen	
Mild hepatomegaly	+
Mild splenomegaly	+
Skeletal	
Mild dysostosis multiplex	−
Ovoid thoracolumbar vertebrae	−
Dense calvaria	−
Mild joint stiffness	−
Skin, nails, and hair	
Hirsutism	−
Coarse hair	−
Neurologic	
Neurologic deterioration, progressive	−
Slowing mental development by 1.5 to 3 y of age	+
Mental retardation	+
Seizures	−
Aggressive behavior	−
Severe behavioral problems at age 3–4 y	−
Laboratory abnormalities	
N-acetyl-α-D-glucosaminidase deficiency in fibroblasts	+
Heparan sulfate excretion in urine	+
Onset in early childhood	−
Some patients have an attenuated phenotype	+

Conclusion

The penetrance of genes in individuals can be variable. As we have seen in our case, the mutation in the *NAGLU* gene produces a slight phenotype in the individual. Therefore, some types of MPS have a mild clinical result, so caution is required. In storage diseases, such as MPS, diagnosis is usually made primarily by enzyme analysis followed by genetic testing. However, in our case, the mutation was first detected by genetic testing and then confirmed by leukocyte enzyme analysis.

Authors' Contributions

M.E. and M.S. conceived and designed the study. A.B. and F. K. performed clinical assessments. B.G. performed experiments, and contributed to data acquisition, analysis, and interpretation. B.G. drafted the manuscript. All authors contributed to critical revision of the manuscript for intellectual content and final approval of the manuscript.

Note

Written informed consent was obtained from the patient's legal guardian(s) for publication of this case report.

Conflict of Interest

None declared.

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