GEORGIAN BIOMEDICAL NEWS

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994 VOLUME 1. ISSUE 1. JANUARY-MARCH 2023

Genetic Heterogeneity of Iduronate-2-Sulfatase in Children Diagnosed with Mucopolysaccharidosis Type II in Azerbaijan

Alizada Sevda Aydin,[1] Aliyeva Kamila Ali Agha,[2] Musayev Shirkhan Aftandil,[3] Rasulov Elkhan Mammad Rasul[3]

DOI: 10.52340/GBMN.2023.01.01.15

ABSTRACT

Background: Mucopolysaccharidosis (MPS) is a rare hereditary disease of lysosomal storage with an X-linked recessive inheritance type, and Hunter's disease is a fairly rare manifestation of mucopolysaccharidosis type II.

Objectives: In the present study, a modern molecular-genetic diagnostic method such as New Generation Sequencing (NGS) was used for the first time to identify iduronate-2-sulfatase (IDS) mutations in the Azerbaijan population.

Methods: In four patients with similar clinical manifestations of Hunter's disease, we carried out enzyme and molecular genetic analysis for all MPS types: MPS I, MPS II, MPS III, MPS IV, and MPS VI.

Results: All four probands observed iduronate-2-sulfatase activity deficiency that corresponded with MPS type II. Molecular genetic analysis of the IDS gene identified three different mutations: -1215del, 1106 (C-G), and 322 (G-T). In consequence of those mutations: - 1215del, 1106 (C-G), and 322 (G-T) appear correspondent amino acid substitution in synthesized protein - enzyme composition: Leu406Phefs*34, Asp358Leu, Tyr108Asp, relatively.

Conclusions: Taking into account the reproductive age of the parents of affected children, further preventive prenatal studies are recommended in the families of the index patients.

Keywords: Enzyme; fluorimetry; gene; Hunter's disease; iduronate-2-sulfatase; lysosomal storage diseases; mucopolysaccharidosis.

BACKGROUND

Mucopolysaccharidosis type II or Hunter syndrome is a rare recessive X-linked genetic pathology of the group of lysosomal storage diseases. Syndrome got its name after Doctor Charles A. Hunter (1873 - 1955) who was the first to describe the disease in 1917.[1,2]

The condition first appears in children between the ages of 2-4 and is characterized by the thickening of the lips, tongue, and hard motions of joints, as well as growth retardation. Upper respiratory obstruction, recurrent rhinitis, and groin and umbilical hernias are all identified as features of the disease.[3]

The rude appearance features (Gargoyles), low pitch gruff voice, and frequent respiratory virus infections are examples of the disease's outer characteristics. Thickened skin, a narrow neck, and gaps in the teeth are frequent symptoms of the illness.[4]

Hunter syndrome occurs due to a series of enzymatic deficiencies, especially deficiency of iduronate 2-sulfatase (IDS) which leads to the storage of protein-hydrocarbon complexes (glycosaminoglycans) and lipids in cells.[5-7]

Iduronate 2-sulfatase (IDS) is the protein synthesized by the eponymous gene and consists of 550 amino acids with a molecular mass of 1873 kDa.[8]

This enzyme takes part in the metabolism of glycosaminoglycans, which are components of connective tissue. Genetic mutations can cause either an enzyme deficiency or to completely block the synthesis of an enzyme, which results in the development of mucopolysaccharidosis type II. IDS gene expresses in the liver, kidney, lungs, and placenta and has nine exons. Iduronate-2-sulfatase, an encoded protein, has two polypeptide chains with masses of 42 kDa and 14 kDa that make up its active form.[8] The enzyme iduronate-2-sulfatase participates in the hydrolysis of glycosaminoglycans in lysosomes and catalyzes the separation of the sulfate group from dermatan sulfates and heparin sulfates.[9]

According to the ClinVar database for 2020, the IDS gene has 265 confirmed harmful mutations. The majority of these alterations are mononucleotide polymorphisms, duplications, and deletions. The third, eighth, and ninth exons of the gene are where point mutations are found.[6] [5]. Recombination between the IDS gene and the pseudogene IDS2, which is positioned closer to the telomere region, has been documented. This recombination hamper mutation analysis.[7] [6]. Mucopolysaccharidosis type II development is related to pathogenic mutations. However, the severity of the disease might vary with the same mutations.[10]

The goal of the current study was to identify MPS II-affected children and screen for IDS gene mutations in the Azerbaijani community.

METHODS

Overall, 4 affected boys of 56 tested patients, aged between six months and eleven years old, were found to have MPS II during initial clinical evaluations at the Central Gyandzha City Hospital and Central Regional Hospitals of the administrative areas of Shamakhi, Guba, Barda, Salyan, Siyazan, Shamkir, and Shirvan and final evaluation at the Children's Republic Clinical Hospital under the Health Ministry of Azerbaijan Republic between 2018-2023 years. The genetic analysis was performed at Centogene Laboratories, Rostock, Germany (Prof. Dr. Rolfs A.) and Genom Clinical Laboratory Baku city, Azerbaijan.

The fluorimetry technique was used for the determination of Iduronate-2-sulfatase enzyme activity. The new-generation sequencing technique was used to examine the IDS gene in a DNA sample taken from the patient's peripheral blood. More than 99% coding areas of those genes have been examined with reading depth no less than 50X. There are 1559 indicators in the average reading depth. Connections exon-intron (± 10np) were included in the analysis. Obtained data pathogeny classification was carried out according to the American College of Medical Genetics and Genomics (ACMG) Guidelines and Standards.[11]

RESULTS

The four affected boys (11 years old M.A. from Shamkir, 5 years old I.F., and 6 months old I.A. from the Siyazan areas of Azerbaijan, and 4 years old B.T. from the Baku city, Mashtagha) displayed distinct clinical signs of MPS II.

We carried out screening of identification of correspondent enzyme activities on all MPS types in all four patients. Table 1 presents an example of enzyme activity indices of patient I.F.

TABLE 1. The MPS enzyme activities screening results for patient I.F.

Enzymes	*Activity (µmol/L/h)*	Cut-off value
Alpha-L-iduronidase	16.8	>1.5
Iduronate-2-sulfatase	0	>2.5
N-acetyl-a-glucosaminidase	5.1	>0.5
N-acetylgalactosamine-6-sulfatase	3.2	>0.2
Arylsulfatase B	10.2	>1.0
Beta-glucuronidase	13.7	>5.0

Table 2 represents the results of Iduronate-2-sulfatase activity analysis in MPS II suspicious patients.

TABLE 2. Iduronate-2-sulfatase activity in MPS II suspicious patients

Patients	*Results*	Reference	Interpretation	Method
M.A.	< 0,8 (LOD) µmol/L/h	≥ 5,6 µmol/L/h	Pathologic	Fluorimetry
I.F.	< 0,0 (LOD) µmol/L/h	≥ 5,6 µmol/L/h	Pathologic	Fluorimetry
I.A.	< 0,0 (LOD) µmol/L/h	≥ 5,6 µmol/L/h	Pathologic	Fluorimetry
B.T.	< 0,8 (LOD) µmol/L/h	≥ 5,6 µmol/L/h	Pathologic	Fluorimetry

Abbreviations: LOD: limit of detection

All four individuals have iduronate-2-sulfatase enzyme deficiencies. Patients M.A. and B.T. showed extremely low activity levels: 0.8 mol/L/h. In I.F. and I.A. brothers, there was absolutely no enzyme activity. We performed a genetic investigation to validate the MPS II diagnosis; the findings are reported in Table 3.

TABLE 3. The results of IDS genetic analysis

Patients	*M.A.*	*I.F.*	*I.A.*	*B.T.*
Variant coordinates	NM_000202.5: c.1215del NM_000202.8(IDS): c.1216_1217del (p.Leu406Phefs*34)	NM_000202.5: c.1106C>G	NM_000202.5: c.1106C>G	NM_000202.5: c.322T>G
Amino acid change	p.(Leu406Phefs*34)	p.(Asp358Leu)	p.(Asp358Leu)	p.(Tyr108Asp)
Snp idetifier	N/A	N/A	N/A	N/A
Zygosity	Hemizygous	Hemizygous	Hemizygous	Hemizygous
In silico parameter	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa : N/A	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster : N/A Conservation_n t: N/A Conservation_a a: N/A	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A Conservation_nt: N/A Conservation_aa: N/A	PolyPhen: Probably damaging Align-GVGD: C15 SIFT: - MutationTaster: Disease causing Conservation_nt: high Conservation_aa: high
Allele frequency	gnomAD: - ESP: - 1000 G: - CentoMD: -	gnomAD: - ESP: - 1000 G: - CentoMD: -	gnomAD: - ESP: - 1000 G: - CentoMD: -	gnomAD: - ESP: - 1000 G: - CentoMD: -
Type and classification	Frameshift Pathogenic (class 1)	Frameshift Pathogenic (class 1)	Frameshift Pathogenic (class 1)	Missense Pathogenic (class 1)

Abbreviations: IDS: identify iduronate-2-sulfatase

DISCUSSION

Hunter syndrome, an X-linked recessive mucopolysaccharidosis type II, is linked to pathogenic mutations in the IDS gene. A lysosomal storage disorder called mucopolysaccharidosis type 2 (MPS II) causes a significant buildup of glycosaminoglycans and many symptoms, such as distinctively coarse facial characteristics, low height, cardio-respiratory dysfunction, and skeletal

Georgian Biomedical News
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

abnormalities. Without neuronal involvement, it appears as a continuum ranging from a severe to an attenuated form (orpha.net ORPHA: 580).

We conducted a screening for each of the four patients to determine the proper enzyme activity. Deficits in the enzyme iduronate-2-sulfatase impact all four individuals.

The results of the enzyme study were validated by genetic analysis of blood samples from M.A. patients. IDS gene mutation was identified as 1215del in hemizygous condition. As a result of the protein-level mutation, the amino acid Leucine was substituted for Phenylalanine at position 406.

The reading frame shift caused by the IDS variation c.1215del p (Leu406Phefs*34) starts at codon 406. The stop codon 33 places downstream marks the end of the new reading frame. According to the Centogene and ACMG recommendations, we classify this variation as pathogenic (class 1) since it has pathologically low enzyme activity and clinical data available.

The IDS gene mutation that replaced the cytosine nucleotide with the guanine at position 1106 was found in the brothers I.F. and I.A. The amino acid leucine is substituted for asparagine at position 358 as a result of the aforementioned mutation at the protein level.

A distinct mutation was found in the IDS gene at position 322 in the case of B.T. This mutation caused the amino acid tyrosine to be replaced by the amino acid asparagine at the protein level. Tyr is changed to Asp at position 108 of the amino acid chain by the IDS variation c.322T>G p.(Tyr108Asp).

CONCLUSIONS

The current study was the first genetic screening of iduronate-2-sulfatase (IDS) in children with mucopolysaccharidosis in the Azerbaijan population. Further preventative prenatal tests are advised in the families of the index patients, taking into consideration the reproductive ages of the parents of the affected children.

AUTHOR AFFILIATION

1 Azerbaijan Medical University, Baku, Azerbaijan;
2 Baku State University, Baku, Azerbaijan;
3 Genome Clinical laboratories, Baku, Azerbaijan.

ACKNOWLEDGMENTS

We are grateful to Prof. Dr. Rolfs A. and his colleagues at the Centogene Laboratory in Rostock, Germany, and our colleagues at the Genom Clinical Laboratory in Baku, Azerbaijan, who made a significant contribution to the current work.

REFERENCES

1. Charles Hunter. A Rare Disease in Two Brothers//Proceedings of the Royal Society of Medicine. April 1917. Volume 10, Issue Study Dis Child, p.104-116. https://doi.org/10/1177/003591571701001833
2. M. Marchione. AP Exclusive: US scientists try 1st gene editing in the body. December 15, 2017.
3. Giugliani R., Federhen A., Michelin-Tirelli K., Riegel M., Burin M. Relative frequency and estimated minimal frequency of Lysosomal Storage Diseases in Brazil: Report from a Reference Laboratory. Genet. Mol. Biol. 2017 Jan-Mar;40(1):31-39. http://doi.org/10.1590/1678-4685-GMB-2016-0268.
4. Gupta A., Utrarilli A., Dalal A., Girisha K.M. Hunter syndrome with late age of presentation: clinical description of a case and review of the literature. BMJ Case Rep., 2015 May 14;2015:bcr2015209303. p.1-3. http://doi.org/10.1136/bcr-2015-209305.
5. Tomatsu S., Lavery C., Lavery Ch., Giugliani R., Harmatz P., Scarpa M., Wegrzyn G., Orii T. Mucopolysaccharidoses Update (2 Volume Set). August 2018. Volume I. Protests Riots Past Present Future Perspect. p.165-209.
6. Rezende M.M., Muller K.B., Pereira V.G., D'Almeida V. Brazilian reference values for MPS II screening in dried blood spots - A fluorimetric assay. Clinical Biochemistry. Volume 47, Issues 13-14, September 2014, p.1297-1299. http://doi.org/10.1016/j.clinbiochem.2014.06.010.
7. Roberts J., Stewart C., Kearney S. Management of the behavioral manifestations of Hunter syndrome. Br J. Nursing, Vol.25, No.1. 15 Jan 2016. p.22-30. http://doi.org/10.12968/bjon.2016.25.1.22.
8. D'Avanzo F., Rigon L., Zanetti A., Tomanin R. Mucopolysaccharidosis Type II: One Hundred Years of Research, Diagnosis, and Treatment. Int. J. Mol. Sci. 2020; Feb 13;21(4):1258. http://doi.org/10.3390/ijms21041258.
9. Happ H., Weh E., Costakos D., Reis L.M., Semina E.V. Case report of homozygous deletion involving the first coding exons of GCNT2 isoforms A and B and part of the upstream region of TFAP2A in congenital cataract. BMC Med. Genet. 2016 Sep 8;17(1):64, p.1-6. http://doi.org/10/1186/s12881-016-0316-0.
10. Chulaluck K., Chalurmpon S., Chupong I., Wechapinan T., Sri-Udomkajorn S., et al., Whole exome sequencing revealed mutations in FBXL4, UNC8o, and ADK in Thai patients with severe intellectual disabilities. Gene. 2019 May 15;696:21-27. http://doi.org./10.1016/j.gene.2019.01.049.
11. American College of Medical Genetics and Genomics. Practice guidelines. https://www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/Practice-Guidelines.aspx.

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994