Molecular Genetics and Metabolism Reports 37 (2023) 101003

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Frequency of iduronate-2-sulfatase gene variants detected in newborn screening for mucopolysaccharidosis type II in Japan

Yusuke Hattori [a,b], Takaaki Sawada [b,c], Jun Kido [b,c,*], Keishin Sugawara [c], Shinichiro Yoshida [d], Shirou Matsumoto [b,c], Takahito Inoue [e,f], Shinichi Hirose [g], Kimitoshi Nakamura [b,c]

[a] Department of Pediatrics, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan
[b] Department of Pediatrics, Kumamoto University Hospital, Kumamoto, Japan
[c] Department of Pediatrics, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan
[d] KM Biologics Co., Ltd., Kumamoto, Japan
[e] Department of Pediatrics, School of Medicine, Fukuoka University, Fukuoka, Japan
[f] Department of Pediatrics, Fukuoka University Chikushi Hospital, Fukuoka, Japan
[g] General Medical Research Center, School of Medicine, Fukuoka University, Fukuoka, Japan

ARTICLE INFO

Keywords:
Enzyme replacement therapy
Mucopolysaccharidosis type II
Iduronate-2-sulfatase
Newborn screening
Hunter syndrome

ABSTRACT

Mucopolysaccharidosis II (MPS II) is an X-linked, recessive, inborn metabolic disorder caused by defects in iduronate-2-sulfatase (IDS). The age at onset, disease severity, and rate of progression vary significantly among patients. This disease is classified into severe or mild forms depending on neurological symptom involvement. The severe form is associated with progressive cognitive decline while the mild form is predominantly associated with somatic features. Newborn screening (NBS) for MPS II has been performed since December 2016, mainly in Kyushu, Japan, where 197,700 newborns were screened using a fluorescence enzyme activity assay of dried blood spots. We diagnosed one newborn with MPS II with lower IDS activity, elevated urinary glycosaminoglycans, and a novel variant of the *IDS* gene. In the future, NBS for MPS II is expected to be performed in many regions of Japan and will contribute to the detection of more patients with MPS II, which is crucial to the early treatment of the disorder.

1. Introduction

Mucopolysaccharidosis II (MPS II; Hunter syndrome; MIM: 309900) is an X-linked recessive inborn metabolic disorder caused by a defect in iduronate-2-sulfatase (IDS) [1,2]. MPS II is a systemic disease affecting multiple organs. Patients with MPS II frequently develop Mongolian spots, hepatosplenomegaly, peculiar facial features, valvular disease, joint contractures, otitis media, respiratory dysfunction, and psychomotor developmental delay [3]. Depending on the presence of neurological symptoms, the disease is classified into severe and mild forms [4,5]. The severe form is associated with progressive cognitive decline, and the mild form is predominantly associated with somatic features.

Enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) are used to treat MPS II [5]. Previously, HSCT was the only effective treatment for central nervous system (CNS) symptoms [6]. Idursulfase beta and pabinafusp alfa were approved as social insurance medicines in 2021 in Japan. Idursulfase beta is administered into the cerebral ventricles through the fixed reservoir [7–9]. Pabinafusp alfa [10–12] is human IDS fused to the C-terminus of the heavy chain of an anti-human transferrin receptor (hTfR) antibody. It can pass through the blood-brain barrier by way of TfR-mediated transcytosis. These ERTs are expected to be effective in treating CNS symptoms. Moreover, a combination of early detection and initiation of treatment is expected to result in more effective therapeutic effects using these ERTs [13,14]. As the external manifestations of MPS II in patients are not apparent except for extensive Mongolian spots and umbilical or inguinal hernias during the neonatal and infancy periods, it is difficult for many clinicians to diagnose MPS II in infants.

In Taiwan [15–19] and a few states [20–23] of the United States, newborn screening (NBS) for MPS II has been performed to detect patients and provide early treatment. Moreover, NBS for MPS II was added to the Recommended Uniform Screening Panel in the United States in 2022 and has been recommended for regular performance [24].

In Japan, the area where NBS for MPS II has been performed has been

* Corresponding author at: 1-1-1 Honjo, Chuo-ku, Kumamoto City 860-8556, Japan.
E-mail address: kidojun@kuh.kumamoto-u.ac.jp (J. Kido).

https://doi.org/10.1016/j.ymgmr.2023.101003
Received 5 May 2023; Received in revised form 19 August 2023; Accepted 20 August 2023
Available online 28 August 2023

expanding in recent years because MPS II is the most common MPS disease in Japan [25] and effective ERTs have been approved. We have conducted NBS for MPS II since December 2016, the earliest in Japan. Here, we report and discuss the frequencies of MPS II and the *IDS* gene variants detected in NBS.

2. Materials and methods

2.1. Study population

A total of 197,700 newborns, 100,561 male and 97,139 female, participated in this study: 88,505 from Kumamoto Prefecture between December 2016 and July 2022, and 109,195 from Fukuoka Prefecture between April 2019 and July 2022. The number of newborns born in these two prefectures accounted for approximately 6% of all newborns born in Japan during these periods. We performed multiplex screening for some lysosomal storage diseases (LSDs; Fabry disease, Pompe disease, Gaucher disease, MPS I, and MPS II) and hypophosphatasia. Female newborns were included in this study. Informed consent was obtained from the parents of 96.2% of all newborns born in these two prefectures. Dried blood spot (DBS) samples were prepared in each maternity clinic or obstetric department as part of a routine public health program using a heel-prick procedure 4–6 days after birth. The blood spots were blotted onto filter paper (Toyo Roshi Kaisha Ltd., Tokyo, Japan) and dried for at least 4 h at room temperature (15 to 25 °C). The samples were sent to the Newborn Screening Center at KM Biologics Co. Ltd. (Kumamoto, Japan) by mail, where public-funded NBS was conducted within 1 week of preparation. The DBS samples were transferred to Kumamoto University to assess the IDS activity.

2.2. NBS program for MPS II

IDS activity was assayed in DBS samples. Newborns with IDS activity below the cutoff level (December 2016 – November 2019: <10 pmol/h/disk, December 2019 – July 2022: <5 pmol/h/disk) were recalled, and DBS samples were prepared again for a second IDS assay. Newborns whose IDS activity was still below the cutoff level were referred to the hospital for clinical assessment, and physical examination and biochemical assays were performed to detect MPS II symptoms. Informed consent was obtained again from the parents after a detailed explanation, and the *IDS* gene of the newborns was sequenced to confirm the diagnosis.

2.3. IDS assay in DBS samples

The IDS enzyme assays were conducted at Kumamoto University from December 2016 to January 2019 using Method I described below. From February 2019, the assays were performed at KM Biologics Co. Ltd. using Method II described below. Totals of 33,258 and 164,250 newborns were screened using Method I and II, respectively.

2.3.1. Method I

The IDS assay for Method I was developed in collaboration with KM Biologics Co. Ltd. and implemented in December 2016. A single disk (3.2 mm in diameter) was punched from the DBS cards and placed into the well of a 96-well plate (Corning Inc., Corning, NY, USA) with 200 μL of extraction solution (0.1% TritonX-100, 0.1% bovine serum albumin, and 0.05% NaN_3 in H_2O). After 1 h incubation at room temperature, a 20 μL aliquot of the extract was transferred to the well of a 96-well black plate (PerkinElmer, Waltham, MA, USA) and 20 μL of substrate solution (0.125 mM 4-methylumbelliferyl-α-L-idopyranosiduronic acid-2-sulfate disodium salt, 5.0 mM cerium acetate, and 0.75 μg/mL recombinant laronidase (Sanofi K.K., Tokyo, Japan) in 50 mM citrate–sodium phosphate buffer, pH 4.5) was added. After 3 h incubation at 38 °C, 200 μL of reaction stop solution (10 mM EDTA in 300 mM glycine/NaOH buffer, pH 10.6) was added. Next, the fluorescence intensity was analyzed at excitation and emission wavelengths of 370 and 465 nm, respectively. Enzyme activity was calculated as picomoles of 4-methylumbelliferone (4-MU) released per hour per disk (pmol/h/disk).

2.3.2. Method II

Method II was performed using an assay kit (Enzaplate LSD; distributed by Siemens Healthcare Diagnostics K.K., Tokyo, Japan) and implemented since February 2019. The extraction, substrate, and stop solutions included in the kit were the same as those used in Method I. This kit, designed for multiplex screening for LSDs and hypophosphatasia, was developed by Daiichi Kishimoto Clinical Laboratories (Sapporo, Japan) and Sapporo Immuno Diagnostic Laboratory Co. Ltd. under the license of KM Biologics Co. Ltd. (see details at intellectual property JP6989872). Briefly, a single 3.2-mm diameter disk punched from a DBS card was incubated in a 96-well plate with 200 μL of extraction solution. In a 96-well black plate, a 20 μL aliquot of the extract was then added to 40 μL of substrate solution and incubated at 38 °C for 3 h, after which 200 μL of reaction stop solution was added. The fluorescence intensity was analyzed at excitation and emission wavelengths of 370 and 465 nm, respectively. The molar product quantities in the assay wells were calculated based on linear regression of the standard curve. The enzyme activity was calculated as picomoles of 4-MU released per hour per disk (pmol/h/disk).

2.4. Molecular analysis of the IDS gene

The *IDS* gene and its flanking regions were amplified by long-range PCR using three forward and reverse primer sets (Supplemental Data 1). The PCR was performed as follows: 94 °C for 2 min, followed by 30 cycles at 98 °C for 10 s and 64.3 °C for 30 s, and 68 °C for 13 min 36 s using KOD FX (Toyobo, Osaka, Japan) and a Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The PCR products (amplicons) were purified using an Agencourt AMP XP PCR Purification Kit (Beckman Coulter, Brea, CA, USA) and quantified with a Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA) using a Qubit 2.0 Fluorometer (Life Technologies). Three amplicons were mixed in equimolar amounts, followed by simultaneous fragmentation and adaptor ligation using a Nextera XT Kit (Illumina, San Diego, CA, USA). The reaction product (library) was validated with a High Sensitivity D1000 ScreenTape (Agilent Technologies, Santa Clara, CA, USA) using an Agilent 2200 TapeStation and quantified using a Qubit dsDNA HS Assay Kit with a Qubit 2.0 Fluorometer to allow for library normalization. Sequencing was performed using a MiSeq Reagent Kit v3 (Illumina) and 150 cycles on a MiSeq sequencer using the "paired-end" sequencing run method. Sequence data analysis, mapping, and variant calling were streamlined using the MiSeq Reporter v2 (Illumina). The reads were aligned to the reference genome sequence of chromosome X (NC_000023.11) using algorithm bwa-0.6.1. Single-nucleotide polymorphism (SNP) and insertion/deletion (INDEL) identification were performed using the Genome Analysis Toolkit (GATK v1.6; Broad Institute, Cambridge, MA, USA). Visualization of the reads was performed using IGV_2.8.0 (Broad Institute, Cambridge, MA, USA) [26].

2.5. Urinary GAGs analysis

Urinary glycosaminoglycans (GAGs) were assayed at a contract lab (SRL, Inc., Tokyo, Japan) using Di Ferrante's method [27] and the carbazole-sulfuric acid method [28] for uronic acid analysis, and using Hopwood's method [29] for high-resolution electrophoresis of GAGs.

2.5.1. Urinary uronic acid analysis

Urine samples (5 mL) were centrifuged at 3000 rpm for 15 min at 25 °C. The supernatant was mixed with 0.2 mL of 10% cetylpyridinium chloride (CPC) in H_2O and incubated at 2–8 °C overnight. The precipitate was collected by centrifugation at 3000 rpm for 15 min at 0 °C, suspended in 5 mL of 1% potassium acetate in ethanol, and incubated at

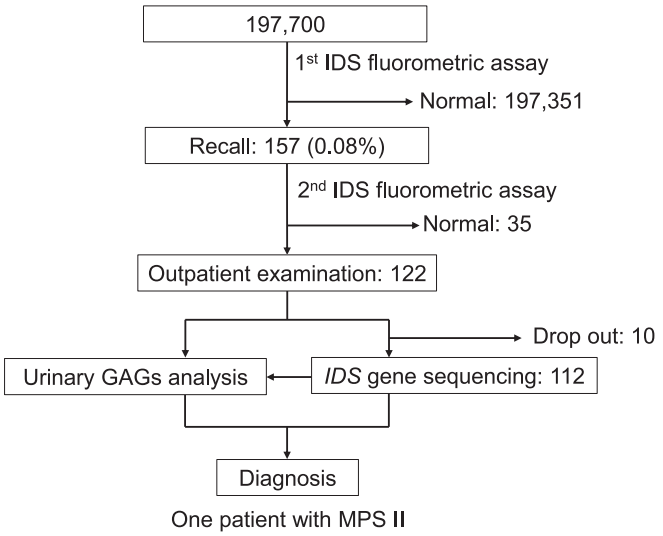

Fig. 1. Flowchart of newborn screening for MPS II.

2–8 °C for 2–3 h. The precipitate was collected again by centrifugation at 3000 rpm for 15 min at 0 °C, dried under a stream of dry nitrogen at 25 °C, and dissolved in 1 mL of water. The dissolved sample (0.1 mL) was mixed with 0.2 mL of 0.125% carbazole in methanol and 2 mL of ice-cold 0.95% sodium borate in concentrated sulfuric acid, heated for 10 min at 100 °C in an oil bath, and cooled to room temperature. The absorbance was measured at 530 nm. A negative control with water and standard, 25, 50 and 100 mg/L of glucuronoloactone were identically treated.

2.5.2. High-resolution electrophoresis of urinary GAGs

Urine samples (2–7 mL), equivalent to 35–100 μg uronic acid, were centrifuged at 3000 rpm for 15 min at 25 °C. The supernatant was mixed with an equal volume of 0.1% CPC in 0.2 M sodium citrate buffer (pH 4.8) and incubated for 30 min at 37 °C. The precipitate was collected by centrifugation at 3000 rpm for 15 min at 0 °C, suspended in 150 μL of 2 M lithium chloride, mixed with 800 μL of ethanol and incubated overnight at 2–8 °C. The precipitate was collected again by centrifugation at 10,000 rpm for 5 min at 0 °C, dried under a stream of dry nitrogen at 25 °C, dissolved in 20 μL of water containing 0.05% phenol red and vortexed vigorously. A part of the dissolved sample equivalent to 5–20 μg uronic acid was loaded at the origin to a cut piece of Titan III Zip Zone cellulose acetate plate (Helena Laboratories, Texas, USA). The sample underwent electrophoresis for 60 min at a constant voltage of 100 V at 15 °C with 1 M barium acetate buffer (pH 5.0), was stained with 0.25% aqueous Alcian blue solution for 15 min, destained in 0.18 M acetic acid, washed in deionized water, and dried at 25 °C. Each channel was scanned using transmission densitometry at 560 nm. The composition of GAGs, heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate 1 (DS1), keratan sulfate and dermatan sulfate 2 were calculated automatically.

2.6. Ethics

This study was approved by the Ethics Committee of Kumamoto University (approval no. 1537). Written informed consent was obtained from the parents or legal guardians of newborns.

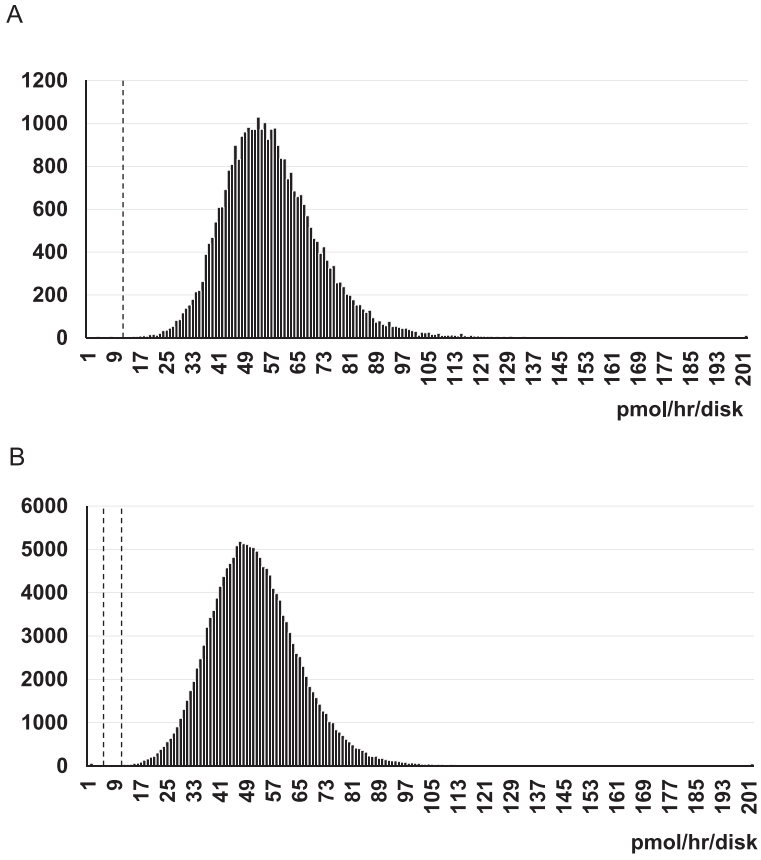

Fig. 2. Histograms of iduronate-2-sulfatase (IDS) activity in newborns. (A) Method I (Dec. 2016–Jan. 2019), $N = 33{,}258$, Median IDS activity: 54.0 pmol/h/disk (IQR, 45.4–64.0). (B) Method II (Feb. 2019–Jul. 2022), $N = 164{,}250$, Median IDS activity: 49.0 pmol/h/disk (IQR, 40.6–58.2). Dashed line: cutoff level.

Table 1
Variants detected in the NBS program for MPS II.

No.	Nucleic acid	Amino acid	Location	ClinVar	Polyphen-2 (Score)	Allele Frequency		References
						ToMMo#	This study$	
1	c.301C > T	p. Arg101Cys	Exon 3	Uncertain significance, benign, likely benign	Probably damaging (1.000)	NA	0.009	Keeratichamroen (2008), Chuang (2018), Chuang (2022)
2	c.418 + 442A > G	–	Intron 3	NR	–	0.024521	0.009	This study
3	c.419-1079 T > C	–	Intron 3	NR	–	NR	0.018	This study
4	c.508-1294G > A	–	Intron 4	NR	–	0.356770	0.509	This study
5	c.684A > G	p.Pro228=	Exon 5	Benign/likely benign	–	0.000348	0.455	Jia (2009), Lin (2020), Bilyen (2020), Chuang (2022)
6	c.708 + 203 T > G	–	Intron 5	NR	–	NR	0.009	This study
7	c.708 + 693C > T	–	Intron 5	NR	–	NA	0.009	This study
8	c.709-263G > A	–	Intron 5	NR	–	0.000015	0.009	This study
9	c.791A > G	p. Tyr264Cys	Exon 6	NR	Probably damaging (1.000)	NR	0.018	This study
10	c.851C > T	p. Pro284Leu	Exon 6	Benign/likely benign	Probably damaging (1.000)	0.000363	0.473	Jia (2009), Kosuga (2016), Chuang (2018), Bilyen (2020), Chuang (2022)
11	c.879 + 1725C > A	–	Intron 6	NR	–	0.024551	0.009	This study
12	c.879 + 1802G > A	–	Intron 6	NR	–	0.003915	0.455	This study
13	c.889C > T	p. Arg297Cys	Exon 7	Likely benign	Probably damaging (1.000)	0.000030	0.118	This study
14	c.934G > A	p. Gly312Ser	Exon 7	Uncertain significance	Probably damaging (1.000)	NR	0.027	Trujillano (2017)
15	c.1003C > A	p. His335Asn	Exon 7	NR	Probably damaging (1.000)	NR	0.009	This study
16	c.1007-1315 T > C	–	Intron 7	NR	–	0.006512	0.009	This study
17	c.1007-1182G > C	–	Intron 7	NR	–	NR	0.009	This study
18	c.1007-1169G > A	–	Intron 7	NR	–	0.008062	0.009	This study
19	c.1007-1089C > T	–	Intron 7	NR	–	0.036030	0.009	This study
20	c.1007-612_610delinsCAA	–	Intron 7	NR	–	NR	0.009	This study
21	c.1180 + 165A > G	–	Intron 8	Benign	–	0.032835	0.009	This study
22	c.1180 + 184 T > C	–	Intron 8	Benign	–	0.396196	0.518	Chuang (2018), Lin (2020), Chuang (2022)
23	c.1180 + 356C > T	–	Intron 8	NR	–	0.000045	0.009	This study
24	c.1180 + 537delC	–	Intron 8	NR	–	NR	0.009	This study
25	c.1180 + 641G > A	–	Intron 8	NR	–	0.363165	0.509	This study
26	c.1180 + 1404C > G	–	Intron 8	NR	–	0.000227	0.009	This study
27	c.1180 + 1756G > A	–	Intron 8	NR	–	0.024702	0.009	This study
28	c.1181–1353A > G	–	Intron 8	NR	–	0.001209	0.455	This study
29	c.1181–1349 T > C	–	Intron 8	NR	–	0.001164	0.455	This study
30	c.1181–1309C > T	–	Intron 8	NR	–	0.007604	0.009	This study
31	c.1394A > G	p. Gln465Arg	Exon 9	NR	Probably damaging (1.000)	NR	0.009	This study
32	c.1478G > A	p. Arg493His	Exon 9	Likely pathogenic, uncertain significance	Probably damaging (1.000)	NR	0.009	Chuang (2018), Chuang (2022)
33	c.1499C > T	p. Thr500Ile	Exon 9	Benign	Probably damaging (0.999)	0.000363	0.318	Chuang (2018), Lin (2020), Bilyen (2020), Chuang (2022)
34	c.*224del	–	Exon 9	Benign	NA	0.107668	0.009	This study
35	c.*1656A > C	–	Exon 9	NR	–	0.036599	0.018	This study
36	c.*2110G > A	–	Exon 9	NR	–	0.107560	0.018	This study
37	c.*3696C > T	–	Exon 9	NR	–	0.071006	0.009	This study

#: Tohoku Medical Megabank (ToMMo 38KJPN-PAR2), $: allele number/110, NA: not available, NR: not registered.

3. Results

3.1. NBS for MPS II

A flowchart and the results of NBS are shown in Fig. 1. In total, 197,700 newborns were screened for IDS activity using DBSs. The median IDS activity was 54.0 pmol/h/disk (interquartile range [IQR]: 45.4–64.0) using Method I, and 49.0 pmol/h/disk (IQR: 40.6–58.2) using Method II (Fig. 2). For Method I and the first 10 months of Method II, the cutoff level was set as 20% of median IDS activity in newborns, 10.0 pmol/h/disk. The recall rates using this cutoff level were almost 0.08% and 0.07%, respectively. From December 2019, the cutoff level was changed to 10% of the IQR, 5.0 pmol/h/disk, to reduce the recall rate, although the recall rate remained 0.08%.

In total, 157 newborns (0.08%) were recalled, requested to prepare DBS again and 122 showed impaired IDS activity in the second IDS activity measurement. All 122 newborns screened positive were male. These 122 newborns underwent physical examinations, biochemical tests, and imaging studies. Of these, 112 newborns continued to *IDS* gene analysis; ten newborns were dropout for *IDS* gene analysis due to lack of

Table 2
Relationships between variants and IDS activity in the DBSs

	variant	N	median	IQR
Method I	c.[684A>G; c.851C>T]	6	3.3	1.8–4.8
	c.889C>T	1	4.9	
	c.1499C>T	8	3.8	2.9–7.7
	Normal newborn controls	**33,243**	**54.0**	**45.4–64.0**
Method II	c.301C>T	1	1.4	
	c.[684A>G; c.851C>T]	44	1.0	1.0–1.4
	c.791A>G	2	1.0	1.0–1.0
	c.851C>T	2	1.2	1.1–1.3
	c.889C>T	12	2.7	2.0–4.4
	c.934G>A	3	5.7	5.2–6.4
	c.1003C>A	1	1.3	
	c.1394A>G	1	1.0	
	c.1478G>A	1	1.0	
	c.1499C>T	27	1.0	1.0–2.4
	Normal newborn controls	**164,183**	**49.0**	**40.6–58.2**

IQR: interquartile range
IDS: pmol/h/disk
Gray: Patients with MPS II.

parental consent (Fig. 1). Urinary GAGs analysis was performed as an outpatient examination or after gene analysis. One newborn with a novel variant of c.1003C > A (p.His335Asn) was diagnosed as a patient with MPS II (Table 1). The frequency of patients with MPS II at the time of screening was 1 in 197,700.

3.2. IDS gene variants detected in this study

This study detected 37 variants of the *IDS* gene that led to lower IDS activity in DBSs (Table 1). Ten variants were exonic, 23 were intronic and the remaining 4 variants were in the 3′ non-coding region. Six of 10 exonic variants, c.301C > T (p.Arg101Cys) [18,30,31], c.684A > G (p.Pro228Pro) [15,23,31,32], c.851C > T (p.Pro284Leu) [18,23,31–33], c.934G > A (p.Gly312Ser) [34], c.1478G > A (p.Arg493His) [18,31], and c.1499C > T (p.Thr500Ile) [15,18,23,31], and one of 23 intronic variants, c.1180 + 184 T > C [15,18,31], have been previously reported as likely pathogenic, likely benign, benign, or uncertain significance. Three variants, c.889C > T (p.Arg297Cys), c.1180 + 165A > G, and c.*224del have been registered in ClinVar as likely benign or benign. The remaining 3 exonic variants, c.791A > G (p.Tyr264Cys), c.1003C > A (p.His335Asn), and c.1394A > G (p.Gln465Arg), were noted. These variants were not detected in the database of the healthy population, were predicted as "probably damaging" by the bioinformatic tool "Polyphen-2", and were not registered in ClinVar. Interestingly, c.684A > G (p.Pro228Pro) always co-existed with c.851C > T (p.Pro284Leu) for the same allele (Supplemental Data 2). Table 2 shows the relationships between the variants and IDS activity in the DBSs. The median value of IDS activity in newborns with these variants was <2.0 pmol/h/disk. The median IDS activity in newborns with c.889C > T (p.Arg297Cys) or c.934G > A (p.Gly312Ser) was >2.0 pmol/h/disk and higher than that in newborns with other variants.

3.3. Urinary GAGs analysis

Fig. 3 shows urinary GAG analysis results for newborns with 10 exonic variants, including c.301C > T (p.Arg101Cys), c.684A > G (p.[Pro228=), c.791A > G (p.Tyr264Cys), c.851C > T(p.Pro284Leu), c.889C > T (p.Arg297Cys), c.934G > A (p.Gly312Ser), c.1003C > A (p.His335Asn), c.1394A > G (p.Gln465Arg), c.1478G > A (p.Arg493His), and c.1499C > T (p.Thr500Ile). The urinary uronic acid levels in newborns with c.1003C > A (p.His335Asn) exceeded the urinary uronic acid levels in the normal control newborns ($N = 9$, 43.3 ± 12.9 mg/g Cre) and were significantly higher than those in newborns with other variants (Fig. 3 and Supplemental Data 3).

3.4. Patient with MPS II detected in this study

This screening program detected one newborn male with MPS II. The primary IDS activity in the patient was 1.3 pmol/h/disk, and secondary IDS activity was 0.1 pmol/h/disk (cutoff 10.0 pmol/h/disk). Outpatient physical examination did not show apparent abnormal findings, except for numerous extensive Mongolian spots on the trunk. IDS analysis revealed a novel variant, c.1003C > A (p.His335Asn). Urinary GAG analysis revealed a high uronic acid level (179 mg/g creatinine). Of these, the composition of DS1, HS and CS in urinary GAGs (36%, 9% and 38%, respectively) indicated a high dermatan sulfate fraction ratio in urinary GAGs (Supplemental Data 3). Based on these results, this patient was diagnosed with MPS II and subjected to ERT using idursulfase (Elaprase®, Takeda Pharmaceuticals Inc., Lexington, MA, USA) from 3 months after birth. His neurocognitive development should be closely monitored and he may require one of the approved therapies to prevent brain disorder progression.

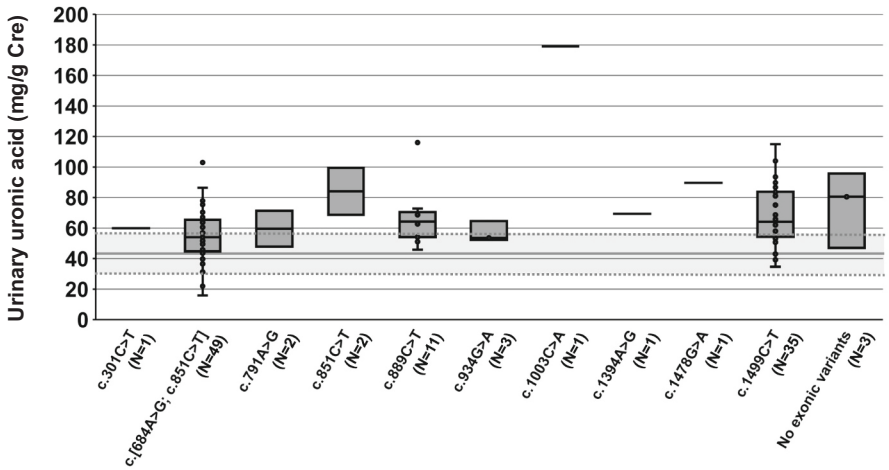

Fig. 3. Relationships of urinary uronic acid levels and variants in newborns receiving *IDS* gene analysis.
The vertical whiskers represent 1.5 times the interquartile range. The gray zone is shown as mean value ± standard deviation of urinary uronic acid levels in normal newborn controls ($N = 9$, 43.3 ± 12.9 mg/g Cre) as indicated by the laboratory (SRL, Inc., Tokyo, Japan).

4. Discussion

This study screened 197,700 newborns and identified one male newborn with MPS II. This frequency was similar to the frequency of MPS II of 1 in 263,158 live births based on a nationwide survey of patients with LSDs in Japan [35].

We previously reported the frequencies of Fabry disease (FD) [36] and Gaucher disease (GD) [37] in Japan based on NBS results. The frequencies of both these diseases were higher than those previously predicted. Although we expected a higher frequency of MPS II than that previously reported [35], the frequency was not significantly different. As patients with MPS II present with many characteristic findings and symptoms, including idiosyncratic facial features and joint contractures, compared to those with FD and GD, pediatricians may be able to diagnose MPS II.

There were differences in the frequency of MPS II in each area: one in 54,862 births in Taiwan [19], one in 73,290 births in Illinois [22], and one in 73,477 births in Missouri [23]. The frequency of MPS II in Japan may be higher than that indicated in this study (1/ 197,700) because this NBS could detect only one patient in a limited area, and this MPS II incidence rate was very low compared to those overseas; more NBS results in expanded areas in Japan should be accumulated.

All patients with MPS II detected by NBS were male; however, in the NBS performed in Missouri, a female newborn with heterozygous c.1499C > T and a female newborn with mildly elevated heparan sulfate levels in the DBS were detected [23]. MPS II has an X-linked inheritance, and it is suggested that heterozygous females also may develop MPS II [38–40].

In this study, 37 *IDS* gene variants were detected in 112 newborns undergoing genetic analysis: 10 exonic variants and 27 intronic variants. Four out of 10 exonic variants were novel, and only c.1003C > A was pathogenic. The most common exonic variants were c.[684A > G and 851C > T], which were identified in 45 patients. This variant is the most frequently detected NBS variant in Taiwan [15,19]. It has also been detected using NBS in Illinois [20–22] and Missouri [23]. Lin et al. followed carriers of this variant (up to 6 years of age) and reported that none of the patients developed MPS II [19]. The second most common exonic variant was c.1499C > T, which was detected in 38 newborns. This variant was also detected in Taiwan, Illinois, and Missouri [19–23]. Lin et al. demonstrated normal IDS activity in all the leukocytes of this variant carrier [19]. In this study, IDS activity in newborns with these 37 variants was as low as that in patients with MPS II. Therefore, clinicians needed to perform outpatient examinations, *IDS* gene analysis, and urinary GAG analysis to identify patients with MPS II. The combination of these clinical examinations may be a psychological and physical burden for newborns with suspected MPS II and their families. The measurement of GAGs using DBS as a 2nd tier test has been performed in Missouri since 2020, which has reduced the false-positive rate [23]. As there may be many false positives in Japan compared to Europe and the United States, the practical use of GAG measurements using DBS may be considered.

5. Conclusions

In this study, 197,700 newborns were screened and 122 showed decreased IDS activity in DBSs. One newborn with a novel variant of c.1003C > A (p.His335Asn) was diagnosed with MPS II using a combination of *IDS* genetic and urinary GAG analyses. The remaining 111 were considered false positives, and many novel variants were detected. As pseudodeficiency variants or VOUS are frequently detected in Japan, it is necessary to introduce GAG measurements in DBSs. This result was obtained in a limited area, and further expansion is needed to estimate the frequency of MPS II in Japan.

Funding

This study was supported in part by a Health and Labor Sciences Research Grant for Research on Rare and Intractable Diseases from the Ministry of Health, Labour and Welfare, Japan (grant number JPMH20FC1025); a Grant-in-Aid for Practical Research Project for Rare/Intractable Diseases from the Japan Agency for Medical Research and Development (AMED; grant numbers JP19ek0109276, JP20ek0109482); and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (Japan Society for the Promotion of Science [JSPS] KAKENHI: grant number JP23K07294, JP23K07316, JP23K14954).

Author statement

YH, TS, JK, and KN designed the study. SY, SM, TS, and TI contributed to measurements and data collection. YH, TS, JK, KS, SY, TI, SM, SH, and KN checked and analyzed the data. YU, TS, JK, and KS wrote the manuscript. JK and KN supervised the study. All authors have read and approved the final manuscript for submission. All authors have agreed to be personally accountable for their contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even those in which the author was not personally involved, were appropriately investigated and resolved, and the resolution documented in the literature.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

Data availability

Data will be made available on request.

Acknowledgments

We are grateful to Ms. Fumiko Nozaki, Ms. Naomi Yano, Ms. Ayuko Tateishi, and Ms. Hiroko Nasu for their technical support related to this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2023.101003.

References

[1] J.J. Hopwood, S. Bunge, C.P. Morris, P.J. Wilson, C. Steglich, M. Beck, E. Schwinger, A. Gal, Molecular basis of mucopolysaccharidosis type II: mutations in the iduronate-2-sulphatase gene, Hum. Mutat. 2 (1993) 435–442, https://doi.org/10.1002/HUMU.1380020603.
[2] S. Verma, S. Pantoom, J. Petters, A.K. Pandey, A. Hermann, J. Lukas, A molecular genetics view on Mucopolysaccharidosis type II, Mutat. Res. Rev. Mutat. Res. 788 (2021), 108392, https://doi.org/10.1016/j.mrrev.2021.108392.
[3] J.E. Wraith, M. Scarpa, M. Beck, O.A. Bodamer, L. De Meirleir, N. Guffon, A. Meldgaard Lund, G. Malm, A.T. Van Der Ploeg, J. Zeman, Mucopolysaccharidosis type II (hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy, Eur. J. Pediatr. 167 (2008) 267–277, https://doi.org/10.1007/S00431-007-0635-4.
[4] J. Holt, M.D. Poe, M.L. Escolar, Early clinical markers of central nervous system involvement in mucopolysaccharidosis type II, J. Pediatr. 159 (2011), https://doi.org/10.1016/J.JPEDS.2011.03.019.
[5] F. D'avanzo, L. Rigon, A. Zanetti, R. Tomanin, Mucopolysaccharidosis type II: one hundred years of research, diagnosis, and treatment, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21041258.
[6] S. Sreekantam, L. Smith, C. Stewart, S. Kearney, S. Lawson, J. Raiman, S. Vijay, S. Santra, Efficacy of early haematopoietic stem cell transplantation versus enzyme replacement therapy on neurological progression in severe hunter syndrome: case

report of siblings and literature review, Mol. Genet. Metab. Rep. 32 (2022), 100881, https://doi.org/10.1016/j.ymgmr.2022.100881.
[7] J. Muenzer, B.K. Burton, P. Harmatz, L.G. Gutiérrez-Solana, M. Ruiz-Garcia, S. A. Jones, N. Guffon, M. Inbar-Feigenberg, D. Bratkovic, M. Hale, Y. Wu, K.S. Yee, D. A.H. Whiteman, D. Alexanderian, Intrathecal idursulfase-IT in patients with neuronopathic mucopolysaccharidosis II: results from a phase 2/3 randomized study, Mol. Genet. Metab. 137 (2022) 127–139, https://doi.org/10.1016/j.ymgme.2022.07.017.
[8] J. Muenzer, B.K. Burton, P. Harmatz, L.G. Gutiérrez-Solana, M. Ruiz-Garcia, S. A. Jones, N. Guffon, M. Inbar-Feigenberg, D. Bratkovic, M. Hale, Y. Wu, K.S. Yee, D. A.H. Whiteman, D. Alexanderian, Long-term open-label extension study of the safety and efficacy of intrathecal idursulfase-IT in patients with neuronopathic mucopolysaccharidosis II, Mol. Genet. Metab. 137 (2022) 92–103, https://doi.org/10.1016/j.ymgme.2022.07.016.
[9] V. Gragnaniello, S. Carraro, L. Rubert, D. Gueraldi, C. Cazzorla, P. Massa, S. Zanconato, A.B. Burlina, A new strategy of desensitization in mucopolysaccharidosis type II disease treated with idursulfase therapy: a case report and review of the literature, Mol. Genet. Metab. Rep. 31 (2022), 100878, https://doi.org/10.1016/j.ymgmr.2022.100878.
[10] R. Giugliani, A.M. Martins, T. Okuyama, Y. Eto, N. Sakai, K. Nakamura, S. So, T. Yamamoto, M. Yamaoka, T. Ikeda, K. Tanizawa, H. Sonoda, M. Schmidt, Y. Sato, Exploration of the efficacy of pabinafusp-alfa (JR-141) on neurocognitive development in hunter syndrome (MPS II): 52-week data from clinical trials in Japan and Brazil, Mol. Genet. Metab. 132 (2021), https://doi.org/10.1016/j.ymgme.2020.12.090.
[11] R. Giugliani, A.M. Martins, T. Okuyama, Y. Eto, N. Sakai, K. Nakamura, H. Morimoto, K. Minami, T. Yamamoto, M. Yamaoka, T. Ikeda, S. So, K. Tanizawa, H. Sonoda, M. Schmidt, Y. Sato, Enzyme replacement therapy with pabinafusp alfa for neuronopathic mucopolysaccharidosis II: an integrated analysis of preclinical and clinical data, Int. J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms222010938.
[12] R. Yamamoto, S. Kawashima, Pharmacological property, mechanism of action and clinical study results of Pabinafusp ALFA (Genetical recombination) (IZCARGO® I. V. Infusion 10 mg) as the therapeutic for Mucopolysaccharidosis type-II (Hunter syndrome), Folia Pharmacol. Japonica. 157 (2022), https://doi.org/10.1254/fpj.21080.
[13] K.S. Yee, D. Alexanderian, Y. Feng, X. Ren, B. Schweikert, O. Ayodele, Impact of the timing of enzyme replacement therapy initiation and cognitive impairment status on outcomes for patients with mucopolysaccharidosis II (MPS II) in the United States: a retrospective chart review, J. Health Econ. Outcomes Res. 9 (2022) 67–76, https://doi.org/10.36469/001c.36540.
[14] P.S. Kishnani, H.M. Amartino, C. Lindberg, T.M. Miller, A. Wilson, J. Keutzer, Timing of diagnosis of patients with pompe disease: data from the pompe registry, Am. J. Med. Genet. A 161 (2013) n/a–n/a, https://doi.org/10.1002/ajmg.a.36110.
[15] H.Y. Lin, R.Y. Tu, S.R. Chern, Y.T. Lo, S. Fran, F.J. Wei, S.F. Huang, S.Y. Tsai, Y. H. Chang, C.L. Lee, S.P. Lin, C.K. Chuang, Identification and functional characterization of IDS gene mutations underlying taiwanese hunter syndrome (Mucopolysaccharidosis type ii), Int. J. Mol. Sci. 21 (2020) 114, https://doi.org/10.3390/ijms21010114.
[16] S.P. Lin, J.H. Chang, G.J. Lee-Chen, D.S. Lin, H.Y. Lin, C.K. Chuang, Detection of hunter syndrome (mucopolysaccharidosis type II) in Taiwanese: biochemical and linkage studies of the iduronate-2-sulfatase gene defects in MPS II patients and carriers, Clin. Chim. Acta 369 (2006) 29–34, https://doi.org/10.1016/j.cca.2006.01.001.
[17] C.K. Chuang, C.L. Lee, R.Y. Tu, Y.T. Lo, F. Sisca, Y.H. Chang, M.Y. Liu, H.Y. Liu, H. J. Chen, S.M. Kao, L.Y. Wang, H.J. Ho, H.Y. Lin, S.P. Lin, Nationwide newborn screening program for mucopolysaccharidoses in Taiwan and an update of the "gold standard" criteria required to make a confirmatory diagnosis, Diagnostics. 11 (2021) 1583, https://doi.org/10.3390/diagnostics11091583.
[18] C.K. Chuang, H.Y. Lin, T.J. Wang, Y.H. Huang, M.J. Chan, H.C. Liao, Y.T. Lo, L. Y. Wang, R.Y. Tu, Y.Y. Fang, T.L. Chen, H.C. Ho, C.C. Chiang, S.P. Lin, Status of newborn screening and follow up investigations for Mucopolysaccharidoses i and II in Taiwan, Orphanet. J. Rare. Dis. 13 (2018) 84, https://doi.org/10.1186/s13023-018-0816-4.
[19] H.Y. Lin, Y.H. Chang, C.L. Lee, Y.R. Tu, Y.T. Lo, P.W. Hung, D.M. Niu, M.Y. Liu, H. Y. Liu, H.J. Chen, S.M. Kao, L.Y. Wang, H.J. Ho, C.K. Chuang, S.P. Lin, Newborn screening program for mucopolysaccharidosis type II and long-term follow-up of the screen-positive subjects in Taiwan, J. Pers. Med. 12 (2022) 1023, https://doi.org/10.3390/jpm12071023.
[20] B.K. Burton, G.E. Hoganson, J. Fleischer, D.K. Grange, S.R. Braddock, R. Hickey, L. Hitchins, D. Groepper, K.M. Christensen, A. Kirby, C. Moody, H. Shryock, L. Ashbaugh, R. Shao, K. Basheeruddin, Population-based newborn screening for mucopolysaccharidosis type II in Illinois: the first year experience, J. Pediatr. 214 (2019) 165–167.e1, https://doi.org/10.1016/j.jpeds.2019.07.053.
[21] B.K. Burton, R. Hickey, L. Hitchins, Newborn screening for mucopolysaccharidosis type II in Illinois: an update, Int. J. Neonatal. Screen. 6 (2020) 73, https://doi.org/10.3390/IJNS6030073.
[22] B.K. Burton, V. Shively, A. Quadri, L. Warn, J. Burton, D.K. Grange, K. Christensen, D. Groepper, L. Ashbaugh, J. Ehrhardt, K. Basheeruddin, Newborn screening for mucopolysaccharidosis type II: lessons learned, Mol. Genet. Metab. 107557 (2023), https://doi.org/10.1016/j.ymgme.2023.107557.
[23] H. Bilyeu, J. Washburn, L. Vermette, T. Klug, Validation and implementation of a highly sensitive and efficient newborn screening assay for mucopolysaccharidosis type II, Int. J. Neonatal. Screen. 6 (2020) 79, https://doi.org/10.3390/ijns6040079.
[24] D.S. Millington, C. Ficicioglu, Addition of MPS-II to the recommended uniform screening panel in the United States, Int. J. Neonatal. Screen. 8 (2022), https://doi.org/10.3390/IJNS8040055.
[25] Y. Yamada, S. Tomatsu, K. Sukegawa, Y. Suzuki, N. Kondo, J.J. Hopwood, T. Orii, Mucopolysaccharidosis type II (Hunter disease): 13 gene mutations in 52 Japanese patients and carrier detection in four families, Hum. Genet. 92 (1993), https://doi.org/10.1007/BF00219675.
[26] J.T. Robinson, H. Thorvaldsdottir, D. Turner, J.P., Mesirov, igv.js: an embeddable JavaScript implementation of the Integrative Genomics Viewer (IGV), Bioinformatics. 39 (2023) 23–24, https://doi.org/10.1093/bioinformatics/btac830.
[27] N.M. Di Ferrante, The measurement of urinary mucopolysaccharides, Anal. Biochem. 21 (1967), https://doi.org/10.1016/0003-2697(67)90087-5.
[28] T. Bitter, H.M. Muir, Analytical biochemistry: a modified uronic acid carbazole reaction, Anal. Biochem. 334 (1962).
[29] J.J. Hopwood, J.R. Harrison, High-resolution electrophoresis of urinary glycosaminoglycans: an improved screening test for the mucopolysaccharidoses, Anal. Biochem. 119 (1982), https://doi.org/10.1016/0003-2697(82)90674-1.
[30] S. Keeratichamroen, J.R.K. Cairns, D. Wattanasirichaigoon, P. Wasant, L. Ngiwsara, P. Suwannarat, S. Pangkanon, J. Kuptanon, P. Tanpaiboon, T. Rujirawat, S. Liammongkolkul, J. Svasti, Molecular analysis of the iduronate-2-sulfatase gene in Thai patients with Hunter syndrome, J. Inherit. Metab. Dis. 31 (2008), https://doi.org/10.1007/s10545-008-0876-z.
[31] C.K. Chuang, Y.R. Tu, C.L. Lee, Y.T. Lo, Y.H. Chang, M.Y. Liu, H.Y. Liu, H.J. Chen, S.M. Kao, L.Y. Wang, H.J. Ho, H.Y. Lin, S.P. Lin, Updated confirmatory diagnosis for mucopolysaccharidoses in Taiwanese infants and the application of gene variants, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/IJMS23179979.
[32] B. Jia, J. Jie Xue, D. Sheng Liang, L. Qian Wu, Molecular analysis of IDS gene and prenatal diagnosis in a Chinese family with mucopolysaccharidosis type II, Zhonghua Er Ke Za Zhi. 47 (2009).
[33] M. Kosuga, R. Mashima, A. Hirakiyama, N. Fuji, T. Kumagai, J.H. Seo, M. Nikaido, S. Saito, K. Ohno, H. Sakuraba, T. Okuyama, Molecular diagnosis of 65 families with mucopolysaccharidosis type II (hunter syndrome) characterized by 16 novel mutations in the IDS gene: genetic, pathological, and structural studies on iduronate-2-sulfatase, Mol. Genet. Metab. 118 (2016) 190–197, https://doi.org/10.1016/j.ymgme.2016.05.003.
[34] D. Trujillano, A.M. Bertoli-Avella, K. Kumar Kandaswamy, M.E. Weiss, J. Köster, A. Marais, O. Paknia, R. Schröder, J.M. Garcia-Aznar, M. Werber, O. Brandau, M. Calvo Del Castillo, C. Baldi, K. Wessel, S. Kishore, N. Nahavandi, W. Eyaid, M. T. Al Rifai, A. Al-Rumayyan, W. Al-Twaijri, A. Alothaim, A. Alhashem, N. Al-Sannaa, M. Al-Balwi, M. Alfadhel, A. Rolfs, R. Abou Jamra, Clinical exome sequencing: results from 2819 samples reflecting 1000 families, Eur. J. Hum. Genet. 25 (2017), https://doi.org/10.1038/ejhg.2016.146.
[35] Y. Koto, N. Sakai, Y. Lee, N. Kakee, J. Matsuda, K. Tsuboi, N. Shimozawa, T. Okuyama, K. Nakamura, A. Narita, H. Kobayashi, R. Uehara, Y. Nakamura, K. Kato, Y. Eto, Prevalence of patients with lysosomal storage disorders and peroxisomal disorders: a nationwide survey in Japan, Mol. Genet. Metab. 133 (2021) 277–288, https://doi.org/10.1016/j.ymgme.2021.05.004.
[36] T. Sawada, J. Kido, S. Yoshida, K. Sugawara, K. Momosaki, T. Inoue, G. Tajima, H. Sawada, S. Mastumoto, F. Endo, S. Hirose, K. Nakamura, Newborn screening for Fabry disease in the western region of Japan, Mol. Genet. Metab. Rep. 22 (2020), 100562, https://doi.org/10.1016/j.ymgmr.2019.100562.
[37] T. Sawada, J. Kido, K. Sugawara, S. Yoshida, S. Matsumoto, T. Shimazu, Y. Matsushita, T. Inoue, S. Hirose, F. Endo, K. Nakamura, Newborn screening for Gaucher disease in Japan, Mol. Genet. Metab. Rep. 31 (2022), 100850, https://doi.org/10.1016/j.ymgmr.2022.100850.
[38] H.Y. Kim, M.J. Kim, M.W. Seong, J.M. Ko, Skewed X-chromosome inactivation in a Korean girl with severe mucopolysaccharidosis type II, Ann. Lab. Med. 42 (2022) 373–375, https://doi.org/10.3343/alm.2022.42.3.373.
[39] A. Jurecka, Z. Krumina, Z. Zuber, A. Rózdzyńska-Światkowska, A. Kłoska, B. Czartoryska, A. Tylki-Szymańska, Mucopolysaccharidosis type II in females and response to enzyme replacement therapy, Am. J. Med. Genet. A 158 A (2012), https://doi.org/10.1002/ajmg.a.34415.
[40] A. Kloska, J. Jakóbkiewicz-Banecka, A. Tylki-Szymańska, B. Czartoryska, G. Weogonekgrzyn, Female hunter syndrome caused by a single mutation and familial XCI skewing: implications for other X-linked disorders, Clin. Genet. 80 (2011), https://doi.org/10.1111/j.1399-0004.2010.01574.x.