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Enzymatic Testing for Mucopolysaccharidosis Type I in Kuwaiti Newborns: A Pilot Study Toward Newborn Screening

Hind Alsharhan [1,2,3,*], Mohammad Z. Haider [2], Bann Qadoura [4], Mariam Ayed [5], Gursev S. Dhaunsi [2] and Hessa Alkandari [1]

[1] Department of Pediatrics, Farwaniya Hospital, Ministry of Health, Sabah Al-Nasser, 92426, Kuwait
[2] Department of Pediatrics, Health Sciences Centre, College of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Postal code 90805, Kuwait
[3] Kuwait Medical Genetics Center, Ministry of Health, Sulaibikhat, 80901, Kuwait
[4] Department of Pediatrics, Amiri Hospital, Ministry of Health, Kuwait
[5] Department of Neonatology, Farwaniya Hospital, Ministry of Health, Kuwait
* Correspondence: hind.alsharhan@ku.edu.kw; hd.alsharhan@live.com

Abstract: Introduction: Mucopolysaccharidosis type I (MPSI) is an autosomal recessive lysosomal storage disorder characterized by deficient or absent α-L-iduronidase (IDUA) enzyme activity due to pathogenic variants in *IDUA* gene. Early treatment with hematopoietic stem cell transplantation and/or enzyme replacement therapy is associated with improved outcomes in this progressive multisystem disease. The diagnosis is usually delayed due to late presentation and nonspecific symptoms resulting in high morbidity and mortality. The incidence of MPSI in US is estimated to be 0.26:100,000, however, it is unknown in Kuwait. This pilot study was undertaken to screen MPSI in all Kuwaiti neonates born at Farwaniya Hospital over a period of 12-months. This study examined the incidence of MPSI in a major center in Kuwait for inclusion in the national newborn screening program to enable its early detection and adequate treatment. **Methods:** All Kuwaiti neonates born at Farwaniya Hospital, Kuwait from December 2021 to December 2022, were screened for MPSI. The screening consisted of determining IDUA enzyme activity in dried blood spots (DBS)-derived samples by Tandem Mass Spectrometry. A follow-up genetic analysis of *IDUA* gene is planned to screen the cases with diminished IDUA enzyme activity as second-tier testing. **Results:** A total of 618 newborns, including 331 (54%) males and 287 (46%) females, were screened. Twenty of them had deficient IDUA enzyme activity but showed negative genetic testing for *IDUA*. However, we have diagnosed one additional female baby with MPSI, who belonged to Farwaniya Hospital, but the parents chose to deliver in a private hospital. She presented at age three months with recurrent upper airway infections, snoring and extensive Mongolian spots. The molecular study revealed previously reported pathogenic nonsense variant in *IDUA* c.1882C>T; p.(Arg628Ter), associated with severe phenotype. That being included, MPSI is estimated to be about 0.3% among tested females and 0.2% of all screened cases in Kuwait. **Conclusion:** Our study is the first to evaluate the incidence of MPSI in Kuwait. Given the single center, small number of screened babies and the short study duration thus far, it is premature to calculate the incidence of MPSI. It is anticipated that as the study continues and more infants are examined, we would be able to estimate the incidence of this disease in our population correctly. Further studies including screening newborns in all maternity hospitals in Kuwait are needed to calculate the actual incidence of MPSI. Our data supports including MPSI in national newborn screening program to allow early initiation of treatment and thus improve disease outcome.

Keywords: mucopolysaccharidosis; lysosomal storage disorder; newborn screening; enzymatic testing; genetic testing

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1. Introduction

Mucopolysaccharidoses type I (MPSI, Hurler Syndrome) is an autosomal recessive subtype of lysosomal storage disorders (LSD), caused by the build-up of glycosaminoglycans (GAG) in the lysosomes secondary to deficiency or absence of α-L-iduronidase (IDUA) enzyme, encoded by *IDUA* gene. The IDUA enzyme is essential for the breakdown of two GAG, heparan sulphate and dermatan sulphate(1–3). The accumulation of lysosomal GAG would result in disturbance of cellular homeostasis leading to progressive cellular damage eventually resulting in a progressive debilitating multisystem disease with variable severity, involving musculoskeletal, cardiac, respiratory, and central nervous systems with poor prognosis in untreated individuals leading to death in the first decade (1,4,5). MPSI has been classified into three different forms based on severity and age of onset: Hurler (severe), Scheie (mild) and Hurler/Scheie (intermediate), with no biochemical differences among them and tendency to be described as having either "severe" or "attenuated" form of MPSI (1,6–8). Infants are typically born with no identifiable clinical symptoms, and based on the severity of the disease, they will be symptomatic within the first three years of life (1,9). MPSI is a pan ethnic disorder affecting males and females equally with incidence of 0.54-1.84 cases per 100,000 newborns (8). Prevalence of severe form is estimated to be 1:100,000 newborns, and attenuated form is estimated 1:500,000 newborns (1). However, due to frequent misdiagnosis and underdiagnosis of diseases, especially the milder forms, it is difficult to determine the true frequency of disease in general population (1,2). Although it is believed that the incidence of inherited genetic disorders in general is more common in Arab countries compared to the other parts of the world due to the high consanguinity rates, there is no reliable data regarding the incidence and prevalence of MPSI in the Middle East and North Africa (MENA) region (10–13). Since the incidence of MPS1 in Kuwait is unknown, this pilot study was carried out to explore the possibility of including it in the national newborn screening program and thus aiding in assessment of its actual incidence and an early diagnosis.

The available treatment options for MPSI include hematopoietic stem cell transplantation (HSCT) and/or enzyme replacement therapy (ERT) (laronidase), which are more effective when administered before the presence of clinical manifestations (1,6,14–18). Many factors have stimulated the efforts to incorporate LSD in the newborn screening (NBS), especially MPSI: 1). The availability of new promising therapies for MPSI and other LSD, 2). The development of new screening tests using dried blood spots (DBS), 3). Early diagnosis and initiation of treatment are known to improve the outcome (1,9,19–21). Like many rare disorders, early diagnosis of MPSI is a challenge. IDUA enzyme activity and GAG accumulation can be measured at birth in DBS and has been adopted by various NBS programs enabling MPSI detection before the appearance of clinical signs and symptoms (9,19,20). Therefore, MPSI NBS was added to the recommended uniform screening panel (RUSP) in the United States in 2016 to be included in the list of disorders that are screened in different states' universal NBS programs (5,22).

MPSI was initially screened with a single-tier approach using IDUA enzyme activity resulted in extremely low positive predictive values due to the low cutoff and pseudodeficiency of IDUA (19). However, a second-tier biomarker was needed for precision and improved positive predictive values, which included measurement of multiple glycosaminoglycans (GAGs) in DBS using MS/MS (9,19). This two-tier screening is suggested to be the gold standard for diagnosing MPSI patients in newborns (9). It is anticipated that subsequent evaluation would include further biomarker studies, thorough clinical assessment, and molecular genetic testing.

This study was undertaken with the aim of estimating the frequency of MPSI in Kuwait and to explore the possibility of including it in the expanded national NBS program especially because of the availability of therapeutic measures (23).

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2. Methods

2.1. DBS Collection Protocol and Screening Population

All Kuwaiti neonates born at Farwaniya Hospital (FH), one of the main six governmental hospitals in Kuwait between December 2021 and December 2022, were included in this study and screened for MPSI. Following the national NBS protocol that was previously described (23), 618 DBS samples were collected within 48-72 hours of life but on a separate Whatman 903 filter card from which other NBS tests were performed. The DBS cards were then gathered, stored at room temperature, and were sent to an analytical laboratory for analysis (ARCHIMED Life Laboratory, Vienna, Austria.

2.2. Ethical Approval

This research study was approved by the Ethical Committee Board at the Ministry of Health, Kuwait as well as the Kuwait University College of Medicine's Institutional Review Board, following the declaration of Helsinki. The study number is MK02/21. Written informed consent for the research study and publication data was obtained from the parents of each newborn.

2.3. Analytical Methods

The screening consisted of quantitating IDUA enzyme activity together with enzyme activities for iduronate-2-sulfatase, alpha-N-acetylglucosaminidase, N-acetylgalactosamine-6-sulfatase, arylsulfatase B and beta-glucuronidase in DBS by tandem mass spectrometry (MS/MS) as previously described (24–27). The cut-off value of IDUA activity is >1.5 μmol/L/h. Blood GAGs quantification was not performed as a second tier-testing.

2.4. Molecular Testing

A follow-up genetic analysis of *IDUA* gene using PCR amplification and sequencing of coding exons 2-14 (exon 10 partly) and flanking intronic regions has been performed on the cases with diminished IDUA enzyme activity as second-tier testing. Genomic DNA was isolated from the same filter card used for enzymatic analysis.

3. Results

A total of 618 newborns, including 331 (54%) males and 287 (46%) females, were screened between December 2021 and December 2022. All the newborns were Kuwaiti descendants. Twenty of them (9 males; 11 females) had deficient IDUA enzyme activity ≤ 1.5 μmol/L/h, ranging from 0.4-1.5 μmol/L/h. However, all had negative genetic testing for *IDUA* resulting in a false-positive rate of 0.03% (Table 1). None of these newborns were heterozygous for variants in *IDUA*. In addition, we diagnosed one female baby with MPSI, who belonged to FH residential area, but the parents chose to deliver in a private hospital instead of FH and thus was not screened at birth. However, she was diagnosed with MPSI at age three-month. That being included, MPSI is estimated to be about 0.3% among tested females and 0.2% of all screened cases in Kuwait, which is a relatively high incidence rate compared to the rest of the world. The delay in analysis of DBS has ranged from 1-week to 10-week-2-day due to delay in shipping the samples to the laboratory for analysis. Turn-around time for the genetic testing for final confirmation ranged from 2 to 11-week (Table 1).

Table 1. Newborns with false positive reduced IDUA enzyme activity. D: day; F: female; IDUA: α-L-iduronidase; M: male; W: week.

Newborn	Sex	Delay in Analysis	IDUA Enzyme Activity (cut-off >1.5 μmol/L/h)	*IDUA* Genetic Testing
1	M	9w4d	1.2	Negative

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2	M	7w4d	0.7	Negative
3	M	6w5d	0.9	Negative
4	M	6w3d	0.4	Negative
5	M	5w6d	1.3	Negative
6	M	8w3d	0.8	Negative
7	M	9w3d	0.5	Negative
8	M	7w4d	1.2	Negative
9	F	6w3d	1.5	Negative
10	M	7w5d	1.3	Negative
11	F	6w3d	0.7	Negative
12	F	7w1d	0.7	Negative
13	F	10w2d	1.3	Negative
14	F	7w	1.0	Negative
15	F	7w3d	0.8	Negative
16	F	6w1d	0.9	Negative
17	F	9w4d	1.1	Negative
18	F	9w2d	0.5	Negative
19	F	8w	1.0	Negative
20	F	8w3d	0.6	Negative

Detailed clinical and diagnostic information of the confirmed Positive Case

The single confirmed MPSI presented at age 3-month-15-day and was diagnosed at that time. MPSI diagnosis was confirmed via both biochemical and genetic testing; enzymatic testing showed reduced IDUA activity at 0.1 µmol/L/h (Cut-off >1.5) and genetic testing revealed a previously reported pathogenic homozygous nonsense variant in *IDUA* (c.1882C>T; p.Arg628Ter). Her clinical examination was notable for facial coarse features, macrocephaly (head circumference >90th percentile) and extensive Mongolian macules over the back, lower and upper extremities. Imaging was remarkable for mild hepatosplenomegaly and dysostosis multiplex; echocardiogram showed mildly thickened anterior mitral leaflet, and aortic valve. She was noticed to have extensive Mongolian macules, noisy breathing, snoring and recurrent upper respiratory tract infections since the age 1-month-old requiring courses of oral antibiotics, inhaled and systemic steroids and inhaled bronchodilators, prescribed by pediatrician and pediatric pulmonologist. She was started on ERT (laronidase) at age 4-months and underwent successful HSCT at age 9-month. She is currently an 18-month-old, doing well and developing adequately.

4. Discussion

Kuwait is a small country located in the Arabian Peninsula, with a population of 4.45 million people of which 1.45 million are Kuwaiti citizens. The vast majority of healthcare services in Kuwait is distributed among six main governmental general hospitals and sixteen private hospitals. Despite the free healthcare services provided by the governmental hospitals, Kuwaiti citizens prefer seeking medical management through the private medical center to reduce the wait time and the convenience of appointments' schedule (28). All Kuwaiti citizens and foreigners have a mandatory Kuwait civil identification card (ID) which allows access to certain governmental services including the governmental hospitals. So, each of the six governmental hospitals are assigned to certain residential area, which is documented in the ID of everyone living in Kuwait.

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Newborn screening in Kuwait has been expanded in 2014 to include 22 endocrine and metabolic disorders with the goal to early detect and treat certain medical conditions to improve the outcome in a cost-effective manner (29). MPSI is currently not included in the Kuwait national NBS program. Disease prevalence and the frequency of carriers in Kuwaiti population are unknown. We have conducted this pilot study in FH, one of the main six governmental hospitals, to evaluate the incidence of the disease in Kuwait and its carrier frequency, as a first step toward including it in the national NBS program.

In this study, 618 Kuwaiti newborns delivered at FH, were screened for reduced IDUA enzyme activity using DBS, followed by DNA sequencing of the *IDUA* gene. We identified 20 neonates with reduced IDUA enzyme activity but negative for biallelic pathogenic variants in *IDUA* gene as second tier testing. None of them were carriers for the disease and unfortunately, total carrier frequency of *IDUA* variant could not be measured in Kuwaiti newborns.

The reduced IDUA activity has resulted in false rate of 0.03% (Table 1) and could be attributed to several factors: 1. the prolonged transfer time to the laboratory for analysis and thus exposure to the heat, 2. pseudodeficiency, or 3. a heterozygous carrier for a pathogenic variant for *IDUA* gene.

We have identified one affected female baby with MPSI at age 3-month-old within the study period that belonged to the study site (FH) but was from recruitment in the study as she was born at a private hospital. Her diagnosis was established based on reduced IDUA activity and positive genetic testing for homozygous pathogenic nonsense variant in *IDUA* (c.1882C>T; p.Arg628Ter), resulting in an incidence of 0.2%. This variant has been associated with severe phenotype of MPSI (Hurler syndrome). It has been frequently reported in homozygous form in affected Kuwaiti individuals and is considered as a founder variant in Kuwait (30,31). The variant (c.1882C>T; p.Arg628Ter) was identified in 14 out of 291 individuals with severe MPSI from Kuwait, accounting for 4.8% of alleles in all individuals (31).

This variant has been previously reported as compound heterozygous with another pathogenic variant in *IDUA* or in homozygous state. There is a close genotype-phenotype correlation among individuals with MPSI (1,32). However, it is well known that the combinations of two null alleles are associated with more severe phenotype, while at least one missense or intronic variant is associated with a milder disease due to some residual enzyme activity. Homozygosity or compound heterozygosity of this nonsense variant (p.Arg286Ter) has been consistently associated with severe phenotype (Hurler syndrome) due to the premature protein truncation of about 50 C-terminal residues resulting in nonfunctional protein (31–37). However, there are two reported individuals with MPSI, one with the same homozygous variant p.Arg286Ter and the other is compound heterozygous for p.Arg268Ter/p.Gln70Ter, both reported with attenuated phenotype (Hurler Scheie), although the phenotype of second case described is consistent with severe disease (Hurler) (38,39).

However, when it is compound heterozygous with a missense variant, it has been associated with attenuated phenotype (Hurler Scheie) or the severe form (Hurler) (40,41). This variant has been previously reported in individuals with MPSI of Middle Eastern origin (Turkey, Tunisia, Saudi Arabia, and Kuwait) (31,35,36,41,42). The early diagnosis of this case has led to early initiation of treatment (ERT) around age 4-month, followed by HSCT at age 9-month with better outcome. This is the first study in Kuwait and the MENA region determining the incidence of MPSI.

Further screening of all Kuwait babies delivered at the other governmental and private hospitals is required to obtain a more accurate incidence of MPSI in Kuwait and facilitate the implementation of a nationwide screening program for MPSI. The results reported in this study support including MPSI in the national NBS program given the severity of the founder mutation and the effectiveness of early management. If MPSI is to be included in the NBS in Kuwait, we recommend obtaining IDUA enzyme activity in DBS, followed by full DNA sequencing of the *IDUA* gene as a second-tier testing. This would not only help in achieving complete genotype of *IDUA* in our population but may also confirm that the nonsense variant (c.1882C>T; p.Arg628Ter) is the most common variant in Kuwaiti population. It will also aid in estimating the carrier frequency for this variant in the Kuwaiti population. Furthermore, it would also allow establishing genotype-phenotype associations and thus improvement in making appropriate therapeutic decisions.

5. Conclusion

This is the first study in Kuwait and MENA region evaluating the incidence and genotype of MPSI via screening asymptomatic newborns. Although none of the 618 screened newborns were confirmed to be affected, we have identified and confirmed MPSI that would have been detected if delivered at FH, resulting in an estimated incidence of MPSI of 0.2% of all screened cases in Kuwait, which is a relatively high incidence rate compared to the rest of the world. Larger cohort is required to have an accurate estimate of the incidence of MPSI in Kuwaiti population and thus enforce incorporating it in the national NBS program because the effective therapeutic measures are available.

Author Contributions: Hind Alsharhan provided clinical evaluations, drafted initial manuscript, and revised the manuscript. Mohammad Z. Haider, Bann Qadoura, Mariam Ayed, Gursev S. Dhaunsi, and Hessa Alkandari critically reviewed, and revised the manuscript.

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Data Availability Statement: All data included in this study can be de-identified and shared upon reasonable request to the corresponding author.

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Conflict of Interest: The authors declare no conflict of interest.

Ethical Approval: This study was performed in accordance with ethical principles for medical research outlined in Declaration of Helsinki. This study was approved by the institutional review board of the Kuwait Ministry of Health and Kuwait Medical Genetics Center.

Informed Consent: Informed consent was obtained from all patients for being included in the study.

References

1. Clarke LA. Mucopolysaccharidosis Type I. GeneReviews® [Internet]. 2021 Feb 25 [cited 2021 Jul 31]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK1162/
2. Mucopolysaccharidosis type I: MedlinePlus Genetics [Internet]. [cited 2021 Jul 31]. Available from: https://medlineplus.gov/genetics/condition/mucopolysaccharidosis-type-i/#frequency
3. Mucopolysaccharidosis Type I - NORD (National Organization for Rare Disorders) [Internet]. [cited 2021 Jul 31]. Available from: https://rarediseases.org/rare-diseases/mucopolysaccharidosis-type-i/
4. Arunkumar N, Langan TJ, Stapleton M, Kubaski F, Mason RW, Singh R, et al. Newborn screening of mucopolysaccharidoses: past, present, and future. J Hum Genet [Internet]. 2020 Jul 1 [cited 2023 May 6];65(7):557–67. Available from: https://pubmed.ncbi.nlm.nih.gov/32277174/
5. Bosfield K, Regier DS, Viall S, Hicks R, Shur N, Grant CL. Mucopolysaccharidosis type I newborn screening: Importance of second tier testing for ethnically diverse populations. Am J Med Genet A. 2021 Jan 1;185(1):134–40.
6. R G, A F, MV R, T V, O A, LL P, et al. Mucopolysaccharidosis I, II, and VI: Brief review and guidelines for treatment. Genet Mol Biol [Internet]. 2010 [cited 2021 Jul 31];33(4):589–604. Available from: https://pubmed.ncbi.nlm.nih.gov/21637564/
7. Zahoor MY, Cheema HA, Ijaz S, Anjum MN, Ramzan K, Bhinder MA. Mapping of IDUA gene variants in Pakistani patients with mucopolysaccharidosis type 1. Journal of Pediatric Endocrinology and Metabolism. 2019;

8. Kemper AR, Brosco J, Comeau AM, Green NS, Prosser LA, Ojodu J, et al. Newborn Screening for Mucopolysaccharidosis Type 1 (MPS I): A Systematic Review of Evidence Report of Final Findings Final Version 1.1 The Condition Review Workgroup. Public Health System Impact-Task Leader. Association of Public Health Laboratories; 2015.
9. Stapleton M, Kubaski F, Mason RW, Shintaku H, Kobayashi H, Yamaguchi S, et al. Newborn screening for mucopolysaccharidoses: Measurement of glycosaminoglycans by LC-MS/MS. Mol Genet Metab Rep [Internet]. 2020 Mar 1 [cited 2023 May 6];22. Available from: https://pubmed.ncbi.nlm.nih.gov/31956510/
10. Bayoumi RA, Yardumian A. Genetic disease in the Arab world. BMJ [Internet]. 2006 Oct 21 [cited 2023 Jun 19];333(7573):819. Available from: https://pubmed.ncbi.nlm.nih.gov/17053218/
11. Al-Gazali L, Hamamy H, Al-Arrayad S. Genetic disorders in the Arab world. BMJ [Internet]. 2006 Oct 21 [cited 2023 Jun 19];333(7573):831–4. Available from: https://pubmed.ncbi.nlm.nih.gov/17053236/
12. Turkia Hb, Abdelmoula M, Chehida Ab, Ben Dridi M, Y -w O R D S KE, Ben Turkia H, et al. Incidence of mucopolysaccharidoses in Tunisia. europepmc.org [Internet]. 2009 [cited 2023 Jun 19];87(n°11):169–72. Available from: https://europepmc.org/article/med/20209839
13. Alsafadi D, Ezzat A, Altamimi F, ElBagoury M, Olfat M, Saleh M, et al. Mucopolysaccharidosis Type I Disease Prevalence Among Patients With Idiopathic Short Stature in Saudi Arabia: Protocol for a Multicenter Cross-sectional Study. JMIR Res Protoc [Internet]. 2021 Aug 1 [cited 2023 Jun 19];10(8). Available from: /pmc/articles/PMC8441598/
14. NA AS, L B, DS B, Y B, C G, N G, et al. Early treatment with laronidase improves clinical outcomes in patients with attenuated MPS I: a retrospective case series analysis of nine sibships. Orphanet J Rare Dis [Internet]. 2015 Oct 7 [cited 2021 Jul 31];10(1). Available from: https://pubmed.ncbi.nlm.nih.gov/26446585/
15. A T, T O, Y S, N S, H T, T S, et al. Long-term efficacy of hematopoietic stem cell transplantation on brain involvement in patients with mucopolysaccharidosis type II: a nationwide survey in Japan. Mol Genet Metab [Internet]. 2012 Nov [cited 2021 Jul 31];107(3):513–20. Available from: https://pubmed.ncbi.nlm.nih.gov/23022072/
16. M A, RF W, PJ O, A O, P V, A F, et al. Long-term outcome of Hurler syndrome patients after hematopoietic cell transplantation: an international multicenter study. Blood [Internet]. 2015 Mar 26 [cited 2021 Aug 2];125(13):2164–72. Available from: https://pubmed.ncbi.nlm.nih.gov/25624320/
17. MH de R, JJ B, AM D, SA J, JH van der L, N M, et al. Enzyme replacement therapy and/or hematopoietic stem cell transplantation at diagnosis in patients with mucopolysaccharidosis type I: results of a European consensus procedure. Orphanet J Rare Dis [Internet]. 2011 [cited 2021 Aug 2];6(1). Available from: https://pubmed.ncbi.nlm.nih.gov/21831279/
18. Laraway S, Mercer J, Jameson E, Ashworth J, Hensman P, Jones SA. Outcomes of Long-Term Treatment with Laronidase in Patients with Mucopolysaccharidosis Type I. J Pediatr [Internet]. 2016 Nov 1 [cited 2021 Aug 2];178:219-226.e1. Available from: http://www.jpeds.com/article/S0022347616307004/fulltext
19. Clarke LA, Dickson P, Ellinwood NM, Klein TL. Newborn Screening for Mucopolysaccharidosis I: Moving Forward Learning from Experience. Int J Neonatal Screen [Internet]. 2020 Nov 19 [cited 2021 Jul 31];6(4):91. Available from: /pmc/articles/PMC7712368/
20. Donati MA, Pasquini E, Spada M, Polo G, Burlina A. Newborn screening in mucopolysaccharidoses. Ital J Pediatr [Internet]. 2018 Nov 16 [cited 2023 May 6];44(Suppl 2). Available from: https://pubmed.ncbi.nlm.nih.gov/30442156/

21. Millington DS, Bali DS. Current State of the Art of Newborn Screening for Lysosomal Storage Disorders. Int J Neonatal Screen [Internet]. 2018 Sep 1 [cited 2023 May 6];4(3). Available from: /pmc/articles/PMC7548896/
22. Recommended Uniform Screening Panel | HRSA [Internet]. [cited 2023 May 6]. Available from: https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp
23. Alsharhan H, Ahmed AA, Ali NM, Alahmad A, Albash B, Elshafie RM, et al. Early Diagnosis of Classic Homocystinuria in Kuwait through Newborn Screening: A 6-Year Experience. Int J Neonatal Screen [Internet]. 2021 Sep 1 [cited 2023 Jun 20];7(3). Available from: https://pubmed.ncbi.nlm.nih.gov/34449519/
24. Scott CR, Elliott S, Buroker N, Thomas LI, Keutzer J, Glass M, et al. Identification of infants at risk for developing Fabry, Pompe, or mucopolysaccharidosis-I from newborn blood spots by tandem mass spectrometry. J Pediatr [Internet]. 2013 Aug [cited 2023 Jun 20];163(2):498–503. Available from: https://pubmed.ncbi.nlm.nih.gov/23465405/
25. Wiesinger T, Schwarz M, Mechtler TP, Liebmann-Reindl S, Streubel B, Kasper DC. α-Mannosidosis - An underdiagnosed lysosomal storage disease in individuals with an "MPS-like" phenotype. Mol Genet Metab [Internet]. 2020 Jun 1 [cited 2023 Jun 20];130(2):149–52. Available from: https://pubmed.ncbi.nlm.nih.gov/32331969/
26. Burlina AB, Polo G, Salviati L, Duro G, Zizzo C, Dardis A, et al. Newborn screening for lysosomal storage disorders by tandem mass spectrometry in North East Italy. J Inherit Metab Dis [Internet]. 2018 Mar 1 [cited 2023 Jun 20];41(2):209–19. Available from: https://onlinelibrary.wiley.com/doi/full/10.1007/s10545-017-0098-3
27. Elliott S, Buroker N, Cournoyer JJ, Potier AM, Trometer JD, Elbin C, et al. Pilot study of newborn screening for six lysosomal storage diseases using Tandem Mass Spectrometry. Mol Genet Metab [Internet]. 2016 Aug 1 [cited 2023 Jun 20];118(4):304–9. Available from: https://pubmed.ncbi.nlm.nih.gov/27238910/
28. Kuwait - Healthcare [Internet]. [cited 2023 Jul 1]. Available from: https://www.trade.gov/country-commercial-guides/kuwait-healthcare
29. Alsharhan H, Ahmed AA, Ali NM, Alahmad A, Albash B, Elshafie RM, et al. Early Diagnosis of Classic Homocystinuria in Kuwait through Newborn Screening: A 6-Year Experience. Int J Neonatal Screen [Internet]. 2021 Sep 1 [cited 2023 Jun 21];7(3):56. Available from: /pmc/articles/PMC8395821/
30. Cobos PN, Steglich C, Santer R, Lukacs Z, Gal A. Dried blood spots allow targeted screening to diagnose mucopolysaccharidosis and mucolipidosis. JIMD Rep [Internet]. 2015 [cited 2023 Jul 1];15:123–32. Available from: https://pubmed.ncbi.nlm.nih.gov/24798265/
31. Ghosh A, Mercer J, Mackinnon S, Yue WW, Church H, Beesley CE, et al. IDUA mutational profile and genotype-phenotype relationships in UK patients with Mucopolysaccharidosis Type I. Hum Mutat [Internet]. 2017 Nov 1 [cited 2023 Jul 1];38(11):1555–68. Available from: https://pubmed.ncbi.nlm.nih.gov/28752568/
32. Clarke LA, Giugliani R, Guffon N, Jones SA, Keenan HA, Munoz-Rojas M V., et al. Genotype-phenotype relationships in mucopolysaccharidosis type I (MPS I): Insights from the International MPS I Registry. Clin Genet [Internet]. 2019 Oct 1 [cited 2022 Jun 3];96(4):281–9. Available from: /pmc/articles/PMC6852151/
33. Chkioua L, Khedhiri S, Kassab A, Bibi A, Ferchichi S, Froissart R, et al. Molecular analysis of mucopolysaccharidosis type I in Tunisia: identification of novel mutation and eight Novel polymorphisms. Diagn Pathol [Internet]. 2011 Apr 26 [cited 2023 Jul 1];6(1):39. Available from: /pmc/articles/PMC3110106/

34. Li P, Wood T, Thompson JN. Diversity of mutations and distribution of single nucleotide polymorphic alleles in the human alpha-L-iduronidase (IDUA) gene. Genet Med [Internet]. 2002 Nov [cited 2023 Jul 1];4(6):420–6. Available from: https://pubmed.ncbi.nlm.nih.gov/12509712/
35. Beesley CE, Meaney CA, Greenland G, Adams V, Vellodi A, Young EP, et al. Mutational analysis of 85 mucopolysaccharidosis type I families: frequency of known mutations, identification of 17 novel mutations and in vitro expression of missense mutations. Hum Genet [Internet]. 2001 [cited 2023 Jul 1];109(5):503–11. Available from: https://pubmed.ncbi.nlm.nih.gov/11735025/
36. Atçeken N, Özgül RK, Yücel Yilmaz D, Tokatli A, Coşkun T, Sivri HS, et al. Evaluation and identification of IDUA gene mutations in Turkishpatients with mucopolysaccharidosis type I. Turk J Med Sci [Internet]. 2016 [cited 2023 Jul 1];46(2):404–8. Available from: https://pubmed.ncbi.nlm.nih.gov/27511503/
37. Coletti HY, Aldenhoven M, Yelin K, Poe MD, Kurtzberg J, Escolar ML. Long-term functional outcomes of children with hurler syndrome treated with unrelated umbilical cord blood transplantation. JIMD Rep [Internet]. 2015 [cited 2023 Jul 1];20:77–86. Available from: https://pubmed.ncbi.nlm.nih.gov/25614311/
38. Venturi N, Rovelli A, Parini R, Menni F, Brambillasca F, Bertagnolio F, et al. Molecular analysis of 30 mucopolysaccharidosis type I patients: evaluation of the mutational spectrum in Italian population and identification of 13 novel mutations. Hum Mutat [Internet]. 2002 [cited 2023 Jul 1];20(3):231. Available from: https://pubmed.ncbi.nlm.nih.gov/12203999/
39. Vazna A, Beesley C, Berna L, Stolnaja L, Myskova H, Bouckova M, et al. Mucopolysaccharidosis type I in 21 Czech and Slovak patients: Mutation analysis suggests a functional importance of C-terminus of the IDUA protein. Am J Med Genet A [Internet]. 2009 May [cited 2023 Jul 1];149A(5):965. Available from: /pmc/articles/PMC3526155/
40. Kwak MJ, Huh R, Kim J, Park HD, Cho SY, Jin DK. Report of 5 novel mutations of the α-L-iduronidase gene and comparison of Korean mutations in relation with those of Japan or China in patients with mucopolysaccharidosis I. BMC Med Genet [Internet]. 2016 [cited 2023 Jul 1];17(1). Available from: /pmc/articles/PMC4983032/
41. Laradi S, Tukel T, Erazo M, Shabbeer J, Chkioua L, Khedhiri S, et al. Mucopolysaccharidosis I: Alpha-L-Iduronidase mutations in three Tunisian families. J Inherit Metab Dis [Internet]. 2005 Dec [cited 2023 Jul 1];28(6):1019–26. Available from: https://pubmed.ncbi.nlm.nih.gov/16435195/
42. Chkioua L, Khedhiri S, Jaidane Z, Ferchichi S, Habib S, Froissart R, et al. [Mucopolysaccharidosis type I: identification of alpha-L-iduronidase mutations in Tunisian families]. Arch Pediatr [Internet]. 2007 Oct [cited 2023 Jul 1];14(10):1183–9. Available from: https://pubmed.ncbi.nlm.nih.gov/17728118/