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Genetic Study of Mucopolysaccharidosis in A Sample of Iraqi Children

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Abstract

Mucopolysaccharidosis (MPS) is a rare heterogenous progressive genetic disorder, which is a subset of lysosomal storage diseases with a consequence of glycosaminoglycans building-up inside the lysosomes which is attributed to specific enzymes absence or deficiency. This study was aimed to identify mutations to be associated with mucopolysaccharidosis in Iraqi children from different centers of metabolic diseases. Concerning the genetic study, the eligible patients whom had no previous genetic analysis, nor had received hematopoietic stem cells transplantation , 75 children were enrolled in this study for molecular confirmation of mucopolysaccharidosis disorders and reporting the existence of any new specific variants among Iraqi patients. Consequently, the study reported various SNPs to be responsible for different MPS types, for instance, there are seven SNPs within IDAU gene of MPS I Iraqi patients, the most frequent one was rs794726877 which accounts for missense C.152G>A. Finally, the study recommends the establishment of awareness programs for affected families regarding these genetic conditions, inheritance pattern and potential availability of reproductive options such as In Vitro Fertilization (IVF), educate the population at risk about the possibility of genetic disease transmission as a result of inbreed marriage and necessity of performing prenatal screening and allowing the option of terminating pregnancy in Iraq for these conditions .

Keywords: Genetic variants, Iraqi children, mucopolysaccharidosis, single nucleotide polymorphism.

Introduction

Mucopolysaccharidosis (MPS) is a rare, progressive genetic disorder, which is a subset of lysosomal storage diseases (LSD) with a consequence of glycosaminoglycans (GAGs) building-up inside the lysosomes which is attributed to specific enzymes absence or deficiency(1). The etiology of each MPS type is attributed to a defect in gene coding for a specific lysosomal enzyme responsible for various GAGs catabolism, for example keratan sulfate (KS), chondroitin sulfate (CS), heparan sulfate (HS) and dermatan sulfate (DS). A wide range of clinical manifestations were presented in MPS patients owning to systemic accumulation of specific GAG in tissues and organs due to lack and / or deficient enzyme activity(2).

Commonly, patients presented with mutual clinical manifestations including, skeletal abnormalities, coarse facial appearance, joint stiffness, short stature, hepatosplenomegaly, loss of vision and hearing disturbance, respiratory and cardiovascular involvement, neurological involvement like mental retardation and cognitive impairment, recurrent infections such as sinusitis or otitis media, frequent hernias and reduced life span. Airway obstruction, infections (systemic or respiratory) and cardiac complications due to building up of GAG in cells, tissues and organs are the chief causes of mortality. Lack or deficiency of different lysosomal enzymes culminate in extensive accumulation of GAGs like DS, HS, KS and CS in lysosomes of variable cells, correspondingly central nervous system (CNS) affected by buildup of monosialic gangliosides (GM2 and GM3) (3). Historically, lysosomes have been known as organelles entirely specific for degradation, however, a dramatic change of this vision about lysosomal function now employed. New evidence about lysosomal function reveals that lysosomes are not only responsible for enzymatic degradation of macromolecules, nonetheless they are implicated actively in crucial cellular pathways, like secretion, signaling process, metabolic regulation, vesicle and plasma membrane trafficking, adaptive immunity, growth and others(4). This new evidence about lysosomal function has interpreted into a better recognition of the LSD pathophysiology with MPS one of the commonest examples owing to failure of

GAGs degradation due to deficient or dysfunction of lysosomal enzymes. Tissues loading with inert substrates thought to be the main culprit of pathology and clinical appearance of MPS, however the new visions regarding lysosomal functions revealing that other major players are involved in MPS pathogenesis. These involve: abnormal composition of cellular membranes irrelevant to accumulation of secondary substrates; trafficking of vesicles, membrane proteins and membranes with aberrant fusion intracellularly; signaling pathways alteration; autophagy impairment; calcium homeostasis abnormalities; oxidative stress, alteration of gene expression and other dysfunctions. The intricacy of the pathogenesis, function and phenotypic appearances of the MPS owing to the defect of these pathways that impacts each other with a complicated interplay[5, 6].

Depending on types of specific GAGs buildup, and the deficiency of definite enzymes, MPS classified into 11 types / subtypes mostly are inherited as an autosomal recessive disorder with the exception of MPS type II inherited as X-linked pattern as illustrated in table-1. Types /subtypes vary in their clinical manifestations, disease course, response to enzyme replacement therapy (ERT) and if irreversible disabilities exist for example in neuropathic types of MPS (MPS IA, II, III, VII)[7]. Various maneuvers have been considered to treat MPS disorders and, for several types/subtypes of MPS, ERT is intended as disease-specific treatment; nevertheless, clinical studies are rapidly moving on the way to other innovative approaches, like substrate reduction therapy (SRT), pharmacological chaperone (PC) and gene therapy. Furthermore, surgical intervention and supportive therapy provide better outcomes[8].

Enzyme replacement therapy (ERT) with human recombinant intravenous infusion is recommended for stabilization of somatic clinical presentations of MPS I, MPS II, MPS IVA, MPS VI and MPS VII, ERT efficacy toward uGAGs declining and reduction of hepatosplenomegaly were reported in long term clinical studies and post-marketing evaluation of ERT. Owing to inability of ERT to access hard to reach some tissues and organs like bones, bronchi, eyes and trachea, thus ERT has poor clinical consequence on these organs. Furthermore, ERT does not pass the blood brain barrier so the impact on central nervous system clinically insignificant and CNS impairments were not reversed by ERT[9].

Table 1. Types / subtypes and enzyme deficiency of mucopolysaccharidosis

MPS type	Inheritance	MPS eponymy	Defective enzyme	Stored GAG(s)
MPS I	AR	Hurler syndrome, Hurler–Scheie syndrome, Scheie syndrome	α-L-iduronidase	heparan sulfate dermatan sulfate
MPS II	XLR	Hunter syndrome	2-iduronate sulfatase	heparan sulfate dermatan sulfate
MPS IIIA	AR	Sanfilippo syndrome A	N-sulfoglucosamine sulfhydrolase	heparan sulfate
MPS IIIB	AR	Sanfilippo syndrome B	α-N-acetylglucosaminidase	heparan sulfate
MPS IIIC	AR	Sanfilippo syndrome C	Acetyl-CoA β glucosaminide acetyltransferase	heparan sulfate
MPS IIID	AR	Sanfilippo syndrome D	N-acetylglucosamine 6-sulfatase	heparan sulfate
MPS IVA	AR	Morquio syndrome A	N-acetylgalactosamine 6-sulfatase	keratan sulfate chondroitin sulfate
MPS IVB	AR	Morquio syndrome B	β-galactosidase-1	keratan sulfate
MPS VI	AR	Maroteaux–Lamy syndrome	N-acetylgalactosamine 4-sulfatase	dermatan sulfate
MPS VII	AR	Sly syndrome	β-glucuronidase	heparan sulfate, dermatan sulfate, chondroitin sulfate
MPS IX	AR	Natowicz syndrome	Hyaluronidase-1	hyaluronic acid
MPSPS	AR	Mucopolysaccharidosis plus syndrome	protein VPS33A	heparan sulfate

Materials and Methods

Patients selection

Patients' inclusive criteria

All pediatric patients were diagnosed with MPS of different types, in which suspected children with obvious clinical presentation were sent to the Lab for measurement of urinary GAGs level followed by determination of enzymes activity from dried blood spot and finally to confirm the diagnosis molecular study should be performed, whether they receive ERT or not.

Patient's age ranged from: newborns to 18-year-old.

Patients' Exclusion Criteria

Heterozygous carriers, patients with other lysosomal storage disease like Gaucher disease and other genetic abnormalities.

Ethical Committee consideration

1. The Scientific and Ethics committee of Mustansiriyah University/ College of Pharmacy accept and approve this scientific research at 28/6/2022 with approval number (1) research number (13).

2. The research committee of Al-Karkh Health Directorate has examined and accept the research protocol as it meets the standards adapted by Ministry of Health for the implementation of research (Ethics Board approval code: 386).

3. Informed patient / parents' consent was obtained after declaration of the scope behind the research. There were no incentives given to the patients.

Study design

An observational prospective cross-sectional study was carried out in Iraq, where the data of MPS patients had been recorded after clinical assessments and biochemical testing. The duration of this study extended from January /2021 to September/ 2022.

Genetic study [10, 11]

The eligible patients who had no previous genetic analysis and who did not receive hematopoietic stem cells transplantation (HSCT) were enrolled in this study for molecular confirmation of MPS disorders and reporting the existence of any new variants among Iraqi patients. Only patients whom registered in Child's Central Teaching Hospital / Metabolic diseases unit / Baghdad and Al-Kadhimiya Teaching Hospital / Metabolic diseases unit / Baghdad were included within the genetic study. Depending on enzymatic activity cut-off of dried blood spot (DBS) sample for the biochemical testing, the patients have or have not genetic analysis, the participants were assigned into four types of MPS as summarized in table- 2 below. The genes were analyzed by an amplicon- based NGS, where the entire coding regions as well as the flanking intronic region were covered.

Table2.Distribution of patients with MPS from child's central / teaching hospital and Al-Kadhimiya Teaching Hospital.

MPS type	Total number of patients	No. of patients with genetic analysis	No. of patients without genetic analysis
MPS I	35	20	14*
MPS II	23	5	18
MPS IIIB	4	4	-
MPS IV A	23	14	9
MPS VI	74	39	34**

* Patients received HSCT and therefore excluded from genetic study.
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DNA Extraction

Miniprep system for blood genomic DNA (gDNA), Promega was used for extraction of gDNA from collected blood samples.

DNA quantitation

According to Promega, determination of the sample quality was done by measuring the concentration of the DNA following the extraction using Quantus Fluorometer. Add 199 µl of diluted Quantifluor dye to 1 µl of DNA then mix and incubate the mixture for 5 minutes at room temperature, DNA concentration were measured subsequently.

Next Generation Sequencing (NGS)

Next generation sequencing technology utilized according to Illumina company workflow, which is performed in five steps: Tagmentation of genomic DNA, post-tagmentation cleanup, amplification of tagmented DNA, cleaning up of the library and check-up of library quality.

Next generation sequencing Data analysis

Raw data (FASTQ) had been aligned with the human reference sequence GRCH38, then the locus of variations was detected using DRAGEN illumina platform and the DRAGEN germline pipeline. Pathogenicity of Single nucleotide polymorphisms (SNPs) obtained from NGS approach were classified into 5 classes: class 1 pathogenic, class 2 likely pathogenic, class 3 variant of uncertain significance (VUS), class 4 likely benign and class 5 which is benign, according to American College of Medical Genetics (ACMG) recommendations and compared with variant pathogenicity in ClinVar of National Center for Biotechnology Information (NCBI).

Results and Discussion

Demographic data and characterization of Mucopolysaccharidosis patients

A total number of 367 patients were included from all metabolic centers in Iraq for

epidemiological study, the preliminary characteristics of all patients were summarized in **table-3**. However, only 159 children were included for genetic study (as illustrated in table-2). The median age of all participated children with MPS was (5± 4.88 year) which was not significantly different across different types of MPS (P=0.314). Distribution among male and female gender participates in the study was 45.5% and 54.5% respectively. Male gender was significantly dominant in MPS II with male: female of 20:1 compared to MPS1, MPS IIIB, MPS IVA and MPS VI where females were the dominant gender 1:1.8, 1:2, 1:1.3 and 1:1.1 respectively. Most of these families were from rural provinces and represent 79.3% while other families reside in urban provinces and represent 20.7%, 82.6% of which were first- and second-degree consanguineous marriage while 17.4% have not consanguinity, 74.2% of these families had positive family history for MPS disease this trend was observed in all MPS types, no significant difference was seen among types.

Patients with no skeletal deformity accounted for only 30 (8.2%) of all cases. By contrast, 46 (12.5%) of the patients had multiple skeletal deformities while the majority 291(79.3%) had one deformity. Scoliosis was the most frequent skeletal deformity observed in 208 (61.9%) of all MPS types followed by kyphosis 60 (17.9%). MPS types were not significantly different in terms of skeletal deformity prevalence.

More than a half of the patients had at least one surgical procedure in the past, only 160 (43.6%) had not underwent surgical procedure to fix a skeletal deformity. MPS I and VI patients underwent more frequent surgeries than other types, yet, that was statistically not significant. Full details are shown in **Table-4**.

A wide variation of demographic data was observed according to the obtained results, as illustrated in table-1 and 2. In this study, the majority of all MPS patients were diagnosed lately at median age 5 $\pm$ 4.88 year, which is inconsistent with the recommendations of previous studies regarding the importance of early diagnosis of MPS patients before the ensue of permanent deteriorations, like somatic, skeletal, and\ or motor. The importance of early diagnosis associated with improved therapeutic outcomes, good prognosis and life expectancy when supportive care is initiated as early as diagnosis is confirmed and prior the onset of manifestations[12-14]. Another study conducted in Netherlands by Gé-Ann Kuipe *et al* also demonstrate a delayed MPS diagnosis[15]. This delay in MPS diagnosis could be attributed to poor implications of newborn screening programs, rural residency of majority of MPS parents, rarity of the disease and ambiguous early symptoms which may be similar to other common disease manifestations and poor disease awareness. Gender distribution by this study reveals dominance of female gender among all types of MPS except MPS II which is X-linked disease that associated with male gender, although other studies reveal male gender predominance of all MPS types over female gender[16, 17] Xueru Chen *et al* show balanced distribution between male and female among all MPS types while excluding MPS II[18]. Current study identifies two females affected by MPS II in Duhok governorate that is inherited as X-linked pattern, this could be attributed to either maternal skewed X-chromosome that is entirely inactivated, or due to monosomy of X-chromosome[19]. The findings of current study demonstrate that most of families of affected children were resides in rural area where consanguineous marriage was rooted as a traditional habit which frequently due to socio-cultural issues, this is consistent with the outcomes of this study that reveal 82.6% of the parents of MPS patients were first and second degree inbreed marriage, and this positively associated with increased frequency of autosomal recessive genetic disorders in such geographical area. similar outcomes were reported in studies carried out by Ekram Fateen *et al* in Egypt[17], Danyah Alsafadi *et al* in Saudi Arabia[20] Ben Turkia *et al* in Tunisia[21], and Varsha Pramodh *et al* in India[22] . Another demographic factor of high concerns is a family history of similar genetic disorders among families of the MPS affected children reported in our study which was 74.2% among total MPS families this result match with the previous study carried out by Jing Zhou *et al* in China[12] and Douglas Colmenares-Bonilla *et al* in Mexico[23], this could be attributed to increased allele carrier frequency in such population due to enclosed nature of that population which encourage inbreed marriage to keep the family structure and property. It is worthy to note that positive family history could be potentially impacting the physicians awareness regarding early screening of other family members[24], prenatal screening[25] and performing carrier confirmatory test to clarify genetic status of this family. Despite the fact that clinical manifestations of different MPS types are heterogenous, numerous manifestations were common among different types of MPS, data of our results reported that majority of patients presented with skeletal deformities in which scoliosis is the commonest orthopedic deformities, likewise more than half of patients had underwent surgical interventions such as hernia fixation. These findings were in accordance with study conducted by Douglas Colmenares-Bonilla *et al* in Mexico[23].

Table-3.Demographic Data of Mucopolysaccharidosis patients

Demographic Data	Gender	Age	Family History	Surgical History	Skeletal Abnormalities	Residence	Consanguinity marriages
Female	200						
Male	167						
≤ 10 years		72					
> 10 years		295					
Positive family history			265				
Negative family history			102				
No surgery				160			
One surgery				146			
Two surgery				50			
> two surgery				11			
No skeletal abnormalities					31		
One skeletal abnormality					268		
Two skeletal abnormalities					54		
> Two skeletal abnormalities					14		
Rural						291	
Urban						76	
No Consanguineous							64
Consanguineous							303

Table 4. Demographics and characteristics of MPS patients according to different types.

Demographics		**Total**		**Type**										**P** value**
				MPS I (n=72)		MPS II (n=42)		MPS IIIB (n=12)		MPS IVA (n=77)		MPS VI (n=163)		
*Age(years) Median ± SD		5 ± 4.88		7.8 ± 6.5		6.9 ± 4.2		9.7 ± 4.0		5.2 ± 4.7		6.3 ± 4.8		0.314
		No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	
Gender	Female	200	54.4	47	65	2	4.8	8	66.7	44	57.1	85	52.2	0.031
	male	167	45.6	25	34.7	40	95.2	4	33.3	33	42.9	78	47.9	
Residence	Rural	291	79.3	57	79.1	31	73.8	12	100	58	75.3	133	81.6	0.264
	Urban	76	20	16	22.2	11	26.2	0	0	19	24.7	30	18	
Consanguinity marriages	No	64	17.4	18	25	7	16.7	2	16.7	17	22.1	20	12.3	0.145
	Yes	303	82.6	55	76.4	35	83.3	10	83.3	60	77.9	143	87.7	
Skeletal Abnormalities	not present	30	8.2	7	9.7	4	9.5	0	0	6	7.8	13	7.9	0.641
	Single	291	79.3	54	75	32	76.2	9	75	61	79.2	135	82.8	
	Multiple	46	12.5	12	16.7	6	14.3	3	25	10	12.9	15	9.2	
Type of skeletal Abnormalities	Scoliosis	208	61.9	45	62.5	24	57.1	8	66.7	38	49.4	93	57.1	0.942
	kyphosis	60	17.9	11	15.3	7	16.7	3	25	13	16.9	26	15.9	
	DSM	58	17.3	13	18.1	8	19.1	2	16.7	16	20.8	19	11.7	
	HD	32	9.5	6	8.3	3	7.1	1	8.3	7	9.1	15	9.2	
	HAF	18	5.4	1	1.4	3	7.1	0	0	5	6.5	9	5.5	
	Knock knee	11	3.3	3	4.2	1	2.4	1	8.3	3	3.9	3	1.8	
Past Surgical Procedures	0	160	43.6	29	40.3	19	45.2	6	50	34	44.5	72	44.2	0.397
	1	146	39.8	33	45.8	12	28.6	6	50	29	37.7	66	40.5	
	2	50	13.6	9	12.5	7	16.8	0	0	13	16.9	21	12.9	
	3	11	3	2	2.8	4	9.5	0	0	1	1.3	4	2.5	

*Independent-Samples Kruskal-Wallis Test used to compare age and ** Chi Square test was used to compare all categoric variables. Abbreviations: DSM, Dysostosis multiplex; HD, Hip dysplasia; HAF, High arch foo

Frequencies and distribution of Mucopolysaccharidosis patients among Iraqi governorates

As Figure 1 shows, MPS was observed in all Iraqi governorates, however the highest prevalence was seen in Basra followed by Najaf and Wasit. Conversely, Ninawa and Muthanna were the governorates with the lowest prevalence of MPS (P <0.001).

MPS IIIB clustering was observed in Basra (58%) and Najaf (17%) while clustering of MPS IVA was observed mainly in Wasit (21%) and Najaf (21%). On the other hand, MPS I was more prevalent in Kirkuk (16%), Basra (14%), Salah -Aldin (11%), and was the only observed type in Ninawa (6%). MPSVI was observed in all governorates except Kirkuk, Ninawa and Al-Qadisiya. North governorates showed clustering of MPSII; 10% of all MPSII cases were observed in Erbil, 10% in Al-Sulaymaniyah and 14% in Dohuk. This type showed other clustering in Karbala (12%), Al-Najaf (14%) and Maysan (12 %) as further detailed in **Figure -1**.

The variability of different MPS frequencies and distribution among Iraqi governorates were reported in this study that could be attributed to the inheritance pattern of the disease as all MPS types inherited by autosomal recessive pattern except MPS II which is X-linked inheritance. Enclosed nature of Iraqi populations, high rate of consanguinity marriage among families resides in rural area of different governorate, little migration rates and founder effect could be attributed to divergence in frequencies of different MPS types by governorates. Such analysis agreed with a study conducted by Yana Puckett *et al (2021)* in USA that reported variability in incidence and frequencies of MPSI by nations[2].

The overall birth prevalence of MPS in the Iraqi population was (2.97 per 100,000 live births), when compared with the birth prevalence of other Arabian countries, such as the UAE (5.5 per 100,000 live births) and Saudi Arabia (15.64 per 100,000 live births), was lower [26]. However, it is similar to the birth prevalence in Tunisia (2.3 per 100,000 live births) [27]. Previous study was published as a part of this study reported that various types of MPS show different frequencies. In the Iraqi population, MPS VI was the most frequently reported type (1.32 per 100,000 live births, representing 44.41% of all MPS cases), followed by MPS IV A and MPS I (0.625 and 0.593 per 100,000 live births, respectively)[28]. The higher frequency rate of MPS VI among other MPS types was also reported in neighboring countries, including UAE and Saudi Arabia, which were 2.51 and 8.0 per 100,000 live births, respectively[29, 30]. Obviously, this could be attributed to the regional founder effect of similar variants or the high consanguinity rate, which increases the frequency of diseased allele transmission, which could be in accordance with the Iraqi situation.

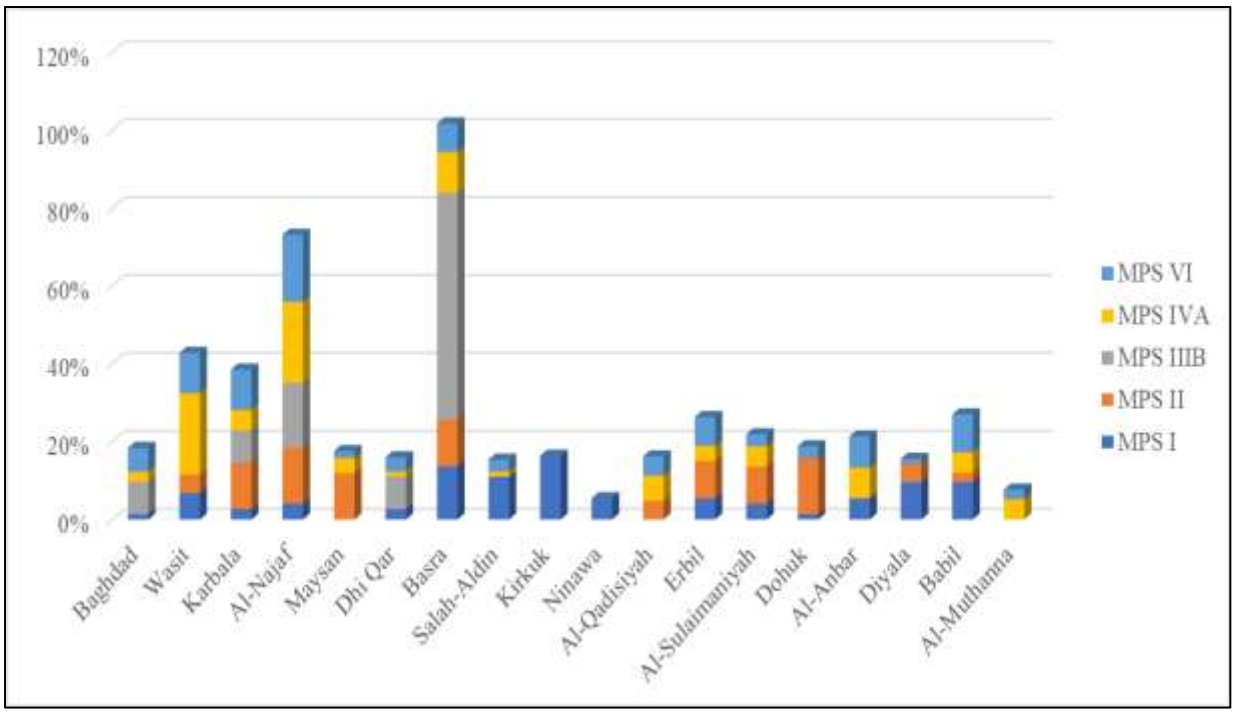

Figure 1. Distribution of MPS patients with various types across Iraqi governorates, Chi square test (P<0.001)

Molecular study of all Mucopolysaccharidosis types and Association between variable SNPs with the demographic and clinical features in different MPS types

Table-5 demonstrates the frequency of SNP ID for each gene with the associated mutation, polymorphism and pathogenicity. Regarding MPS I there are seven SNPs in IDUA gene had been demonstrated through NGS, the most frequent was the missense mutation of rs794726877 identified in 8 out of 34 (23.5%) MPS I patients. NGS data analysis reveals nine SNPs in IDS gene regarding MPS II, the most frequent were the frameshift rs2089504895 and nonsense rs864622772 each accounted for 4 (17.4%). There are only four cases of MSP IIIB had been analyzed by NGS and the result showed only one SNP related to NAGLU gene and the type of mutation was missense. While, NGS analysis for MPS IVA patients showed five SNPs in GALNS gene, the most frequent was a nonsense rs372893383 seen in 8 (34.8%) of the patients. Finally, data analysis of MPS VI patients showed the highest molecular heterogeneity with 13 different SNPs in ARBS gene. Nonsense, rs765711776, was the most frequent SNP seen in 23 (31.5%) of the MPS VI patients. Molecular approaches that implicated to identify disease associated SNPs can be expensive and time consuming. However, molecular studies were mandate not only to confirm MPS diagnosis but also to predict potential associations between genotypes and phenotypes. Generally, there was significant differences between SNPs in various MPS types and patient genders. Most of the SNPs were exclusively observed in one gender rather than the other. In MPS I, for instance, the 4th and 7th SNPs were seen only in females while 2nd and 5th SNPs were seen exclusively in males ($P<0.001$). While, MPS II which inherited as X-linked inheritance and consequently affect only male patients who were enrolled in molecular study therefore all SNPs had been demonstrated in males. Likewise, NGS data analysis for MPS III B patients reveal only one SNP which is more frequent in females. Regarding MPS IV A patients, the 1st SNP was seen only in males while the 3rd SNP has been demonstrated only in females. Similarly, NGS data analysis of MPS VI patients reveals exclusive association of 3rd SNP with females and 5th SNP with males' gender. In terms of consanguinity marriage, all SNPs in MPS I, II, IIIB and IVA were more frequently expresses in consanguinity marriage. The only significant difference was observed in MSP VI ($P=0.042$), five out of nine (55%) of rs771296632 were not associated with consanguinity marriage.

Likewise, most of the SNPs in MPS I, MPS IVA and MPS VI were frequently observed in patients from rural areas. Within MPS II, however, three SNPs (the 1st, 4th and 5th were more frequently observed in patients form urban residence ($P=0.018$). It is worth noting that all patients with rs1554032099 in MPS IIIB were from urban residence. No significance difference ($p > 0.05$) observed between SNPs of different MPS types concerning to skeletal deformities and number of surgical operations performed. Analysis of this study document that various SNPs are responsible for different MPS types, for instance, there are seven SNPs within IDAU gene of MPS I Iraqi patients, the most frequent one was rs794726877 account for missense c.152G>A (p.Gly51Asp) mutation which identified in 8 out of 34 patients (23.5%). This SNP was previously reported in cohort study conducted by Francesca Bertola *et al* in Europe[31], while W402X, Q70X, and P533R were most frequent mutations of IDUA genes identified in other countries[32]. On the other hand, NGS data analysis reveals nine SNPs in IDS gene regarding MPS II, the most frequent were the rs2089504895 resulting in c.133del (p.Asp45fs) frameshift and nonsense rs864622772 which are novel mutations previously unidentified according to alignment in ClinVar. This result was not harmonized with previous studies that reported different SNPs of IDS gene which are more predominant in MPS II patients[33-35], this could be attributed either to ethnic diversity or due difference in biogeographical distributions of mutant allele. Five SNPs were reported by NGS when analyze GALNS gene of MPS IVA patients, rs372893383 was found in the probands responsible for c.1559G>A (p.Trp520Ter), previously this variant was also reported through the study conducted by Mugen Terzioglu *et al* in Turkey[36], Aleksandra Jezela-Stanek *et al* in Central- Eastern Europe[37] and Dengmin He *et al* in South China[38]. Finally as mentioned in previous literature the most frequent type of MPS among Iraqi population was MPS VI which associated with the highest genetic heterogeneity of ARSB gene where NGS data alignment document 13 different SNPs, the most frequent was rs765711776 accounts for the c.753C>G nucleotide change, this result accordant with previously published study by Nouriya Abbas Al-Sannaa *et al* [39] in Saudi Arabia that reported c.753C>G nucleotide change was more frequent among families resides in eastern Saudi provinces. The probands of specific nucleotide changes of all genes that responsible for enzymatic activity could be due to the fact that most of MPS children are off-springs and siblings within the same family therefore they were carrying the same disease-associated mutation or due to high rate of consanguineous marriage between those enclosed population which result in founder effect among these provinces.

The other factors that reported in this study were the association between variable SNPs and demographic and clinical features among all MPS types, and the analyzed data reveals that sex-related distribution of specific SNPs was significant. For instance, in MPS I the 4th and 7th SNPs were seen only in females while 2nd and 5th SNPs were seen exclusively in males ($P<0.001$) as mentioned previously in table 3-7a. The reason of this distribution is not well known, but possibly could be explained by genders variability of encoding genes in sex-chromosome. A retrospective study conducted by Elena M. Varoni *et al* in Italy[40] reports a gender-difference regarding allelic distribution of human β T-cell receptors' SNPs in Caucasian population which is consistent with our outcomes.

Table5. Genetic study for each MPS type.

Type	Mutated gene	SNP ID	Mutation	No (%)	Chr	polymorphism	zygosity	type of mutation	pathogenicity
MPS I	IDUA	rs1389029860	c.439C>T	4 (11.8)	4	p.Gln147Ter	homo	nonsense	pathogenic
		rs1421520718\rs758452450	c.156C>G \ c.223G>A	2 (5.9)	4	p.Phe52Leu \ p.Ala75Thr	hetro	missense\ missense	VUS \ pathogenic
		rs1713799895	c.141G>A	6 (17.6)	4	p.Trp47Ter	homo	nonsense	pathogenic
		rs727503966	c.164dup	5 (14.7)	4	p.Leu56fs	homo	frameshift	pathogenic
		rs753767675	c.2T>C	3 (8.8)	4	p.Met1Thr	homo	missense	pathogenic
		rs780615798	c.385+1G>C	6 (17.6)	4		homo	splice donor	pathogenic
		rs794726877	c.152G>A	8 (23.5)	4	p.Gly51Asp	homo	missense	pathogenic
	Total			**34 (100)**					
MPSII	IDS	rs113993953	c.884A>T	2 (8.7)	10	p.Lys295Ile	homo	missense	pathogenic
		rs1602734459	c.984del	3 (13)	10	p.Ile329fs	homo	frameshift	pathogenic
		rs193302912	c.935G>A	1 (4.3)	10	p.Gly312Asp	homo	missense	pathogenic
		rs193302913	c.908_909del	3 (13)	10	p.Ser303fs	homo	frameshift	pathogenic
		rs199422231	c.1402C>T	3 (13)	10	p.Arg468Trp	hemi	missense	likely pathogenic
		rs2089307950	c.1234G>T	2 (8.7)	10	p.Gly412Ter	homo	nonsense	pathogenic
		rs2089378420	c.1006+2T>G	1 (4.3)	10		homo	splice donor	pathogenic
		rs2089504895	c.133del	4 (17.4)	10	p.Asp45fs	homo	frameshift	pathogenic
		rs864622772	c.1393C>T	4 (17.4)	10	p.Gln465Ter	homo	nonsense	pathogenic
	Total			**23 (100)**					
MPSIII B	NAGLU	rs104894596	c.1444C>T	4 (100)	17	p.Arg482Trp	homo	missense	likely pathogenic

Type	Mutated gene	SNP ID	Mutation	No (%)	Chr	polymorphism	zygosity	type of mutation	pathogenicity
MPS IV A	GALNS	rs118204439	c.1417C>T	5 (21.7)	16	p.Gln473Ter	homo	nonsense	pathogenic
		rs118204446	c.935C>G	1 (4.3)	16	p.Thr312Ser	homo	missense	pathogenic
		rs1909832718	c.1365-1G>C	6 (26.1)	16		homo	splice acceptor	pathogenic
		rs372893383	c.1559G>A	8 (34.8)	16	p.Trp520Ter	homo	nonsense	pathogenic
		rs773746427	c.1364+1G>A	3 (13)	16		homo	splice donor	pathogenic
	Total			**23 (100)**					
MPS VI	ARSB*	rs1554032099	c.281C>A	1 (1.4)	5	p.Ser94Leu	homo	nonsense	likely pathogenic
		rs1554069793	c.1261G>T	6 (8.2)	5	p.Glu421Ter	homo	nonsense	pathogenic
		rs1554079320	c.962T>C	9 (12.3)	5	p.Leu321Pro	homo	missense	pathogenic
		rs376749		1 (1.4)	5				
		rs431905495	c.1143-1G>C	7 (9.6)	5		homo	splice site acceptor	pathogenic
		rs4703764		1 (1.4)	5				
		rs555785323	c.1350G>C	5 (6.8)	5	p.Glu421Ter	homo	missense	pathogenic
		rs727503809	c.944G>A	3 (4.1)	5	p.Arg315Gln	homo	missense	pathogenic
		rs765711776	c.753 C>G	23 (31.5)	5	p.Tyr251Ter	homo	nonsense	pathogenic
		rs768802200	c.323G>T	3 (4.1)	5	p.Gly108Val	homo	missense	VUS
		rs771296632	c.1208C>G	9 (12.3)	5	p.Ser403Ter	homo	missense	VUS
		rs773492223	c.979C>T	2 (2.7)	5	p.Arg327Ter	homo	missense	pathogenic
		rs891298440	c.943C>T	3 (4.1)	5	p.Arg315Ter	homo	nonsense	pathogenic
	Total			**73 (100)**					

*One case was excluded. Abbreviations: Chr, chromosome; VUS, variant of unknown significance, these abbreviations are genes coding for their relevant enzymes GALNS=galactosamine (N-acetyl)-6-sulftase, IDS= Iduronate-2-sulftase ,NAGLU= N-acetyl-alpha-glucosaminidase ,ARSB= arylsulftase B.

Conclusion

The findings of the current study report that late age of diagnosis of all MPS patients at median age 5 ± 4.88 year, females were the dominant gender among all MPS types with highest rate of consanguinity marriage regarding MPS families, therefore it is highly recommended to highlight the importance of Newborn Screening Programs (NSP) for early diagnosis of rare genetic diseases for early commencing of therapeutic interventions whether HSCT or ERT. Various SNPs are responsible for different MPS types, for instance, there are seven SNPs within IDAU gene of MPS I Iraqi patients, the most frequent one was rs794726877 which accounts for missense c.152G>A. Sex-related distribution of specific SNPs was significant. For instance, in MPS I the 4th and 7th SNPs were seen only in females, while 2nd and 5th SNPs were seen exclusively in males ($P<0.001$).

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Ethics Statements

This study was approved by the Scientific and Ethics committee of Mustansiriyah University/ College of Pharmacy accept and approve this scientific research at 28/6/2022 with approval number (1) research number (13).

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(دراسة وراثية لداء عديد السكاريد المخاطي في عينة من أطفال العراق)

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الخلاصة

داء عديد السكاريد المخاطي هو اضطراب وراثي تقدمي متغاير نادر ، وهو مجموعة فرعية من أمراض التخزين الليزوزومية نتيجة تراكم الجليكوزامينوجليكان داخل الجسيمات الحالة التي تُعزى إلى غياب أو نقص أنزيمات معينة. هدفت هذه الدراسة إلى تحديد الطفرات المرتبطة بداء عديد السكاريد المخاطي لدى الأطفال العراقيين من مراكز مختلفة للأمراض الاستقلابية. فيما يتعلق بالدراسة الوراثية، تم تسجيل ٧٥ طفلاً في هذه الدراسة للمرضى المؤهلين الذين لم يكن لديهم تحليل جيني سابق ولم يتلقوا زرع الخلايا الجذعية المكونة للدم، من أجل التأكيد الجزيئي لاضطرابات عديد السكاريد المخاطي والإبلاغ عن وجود أي متغيرات محددة جديدة بين المرضى العراقيين. وبالتالي، ذكرت الدراسة أن العديد من تعدد الأشكال هو المسؤول عن أنواع مختلفة من داء عيديد السكاريد المخاطي، على سبيل المثال، هناك سبعة تعددات SNP ضمن جين IDAU للمرضى العراقيين MPS I، وأكثرها شيوعاً كان rs794726877 والذي يمثل خطأ C.152G>A. وأخيرًا، توصي الدراسة بإنشاء برامج توعية للأسر المتضررة فيما يتعلق بهذه الحالات الوراثية ونمط الوراثة والتوافر المحتمل لخيارات الإنجاب مثل التخصيب في المختبر (IVF)، وتثقيف السكان المعرضين للخطر حول إمكانية انتقال الأمراض الوراثية نتيجة لزواج الأقارب وضرورة إجراء فحص ما قبل الولادة وإتاحة خيار إنهاء الحمل في العراق لهذه الحالات.

الكلمات المفتاحية : المتغيرات الجينية، الأطفال العراقيون ، داء عديد السكاريد المخاطي ،تعدد أشكال النوكليوتيدات المفردة.