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Mucopolysaccharidosis type I - Clinical and genetic characteristics of Romanian patients

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Abstract

***Background**: Mucopolysaccharidosis type I (MPS I) is an autosomal recessive lysosomal storage disorder caused by a deficiency of α-L-iduronidase (IDUA), which leads to the accumulation of partially digested glycosaminoglycans (dermatan sulfate and heparan sulfate) in the lysosomes and induces multisystemic alteration. Hurler (severe), Scheie (mild), and Hurler/Scheie (intermediate) syndromes are clinical subtypes of MPS-I. To date, more than 290 IDUA mutations have been reported. The purpose of this study was to present the clinical and genetic characteristics of Romanian MPS I syndrome patients and their genotype-phenotype correlation.*
***Patients and methods**: Seven patients (5 girls and 2 boys) with MPS type I, belonging to 4 unrelated families, aged 0,75-17.9 years, were enrolled. The study methods consisted in: clinical and standard auxological assessment, bone radiographs, joint ultrasonography, goniometry, neurological and psychological evaluation, hepatic and splenic ultrasonography, cardiological evaluation, otorhinolaryngology examination, ophthalmological examination, spirometry, α-L-iduronidase enzyme activity assay and molecular analysis. **Results**: The seven patients originated from 4 unrelated families, three patients with severe, two patients with intermediate and two with attenuated clinical phenotype. Each patient presented the classical picture of MPS type I picture, represented by: variable coarse facial features, arthropathy, hepatosplenomegaly, cardiac involvement, respiratory dysfunction and neurological impairment. Five patological variants, three point mutations (p.Q70 *, p.I238Q and p.K324R), two deletion c.1045_1047delGAC, c.46_57delTCGCTCCTG) and an insertion (c.1389 insC) were identified in both alleles of the ADUA gene in homozygous or heterozygous form. Two novel mutations (p.K324R and c.1389 insC) were reported. The p.Q70*(c.208C>T) variant was identified in 2 families with severe form of disease (Hurler syndrome) in homozygous status in one family and in compound heterozygous status in the other family.*
***Conclusion:** The p.Q70* missense variant was the most frequent, correlated in all the cases who presented it with severe form, Hurler syndrome, the other mutations being usually isolated and particular for each patient, associated in our patients with less severe MPS I phenotype, as Hurler-Scheie or Scheie syndrome. The results of this study indicated the mutational heterogeneity of the IDUA gene and the difficulty to indicate some correlation between the genotype and phenotype in MPS I patients.*

***Keywords:** MPS I, IDUA, mutations*

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Introduction

Mucopolysaccharidosis type I (MPS I) is a monogenic disease determined by α-L-iduronidase (IDUA) deficiency. IDUA is a lysosomal enzyme involved in glycosaminoglycans (GAGs) degradation, IDUA deficiency having as consequence GAGs accumulations in different organs and tissues, thus leading to multisystemic involvement (1). The disease has three major clinical subtypes. Hurler syndrome is a severe subtype (MPS IH; 607014) characterized by infantile onset, frequent upper respiratory-tract infections, severe organomegaly, progressive skeletal dysplasia (dysostosis multiplex), reduction of linear growth and intellectual disability. The classical picture of intermediate Hurler–Scheie syndrome (MPS IH/S; 607015) with a clinical onset in childhood is represented by severe organomegaly, skeletal involvement and mild/moderate intellectual disability. Scheie syndrome (MPS IS; 607016) is less severe and is clinically characterized by: later onset, visceral and skeletal disease with progressive articular signs, cardiorespiratory involvement, with a psychomotor development which may be normal in early childhood (2). The gene encoding alpha-L-iduronidase (IDUA) has 19 kb, is located on the chromosome 4p16.3 and contains 14 exons and 13 introns. It encodes a precursor IDUA protein of 653 amino acids, which is glycosylated and then processed to the mature form (3-5). The IDUA gene alterations include small gene alterations missense/nonsense, splicing, small deletions, small insertions, and large gene deletions, and complex rearrangements (Froissart et al. 1993). More than 290 distinct pathogenic variants of the IDUA gene have been described in the Human Genome Mutation Database (www.hgmdtrial.biobase-international.org 2019). Most of the identified mutations are point mutations (52%) or small deletions (17%). The two most common IDUA mutations reported in patients from Europe and North America are p.W402X and Q70X and their frequency are around 50% of alleles (6-8). The other part of patients are carrying ”private” mutations (7, 9). Genetic testing of the MPS I patients is useful for the identification of specific genotypes, to indicate genotype-phenotype correlations and also to indicate a correct reproductive option in prenatal diagnosis.

The aim of this research was to study the phenotype and genotype features of the Romanian patients with MPS I syndrome.

Patients and methods

In this study we investigated seven patients with MPS type I, five girls and two boys. They belonged to four unrelated families and their age at diagnosis was between 0.75 and 17.9 years. The enrolment period for this study was between 2005 and 2018 and these patients represent all the patients diagnosed in Romania within this time interval. The study protocol had the approval of the Ethics Committee of Clinical Emergency Hospital for Children, Cluj-Napoca (no. 692/date 27.01.2014). For each patient a written and informed consent to participate at this study was obtained from their parents or legal guardians (10).

The patients were clinically evaluated including the following: auxologic measurements (using www.who.int/childgrowth/standards, Seca Vogel and Halke Hamburg 702 device), goniometry, radiology (skeletal assessment), ultrasonographic, neurological, psychological, otorhinolaryngological (including audiogram), ophthalmological, cardiological (ECG, Doppler echocardiography) evaluations and spirometry. Ultrasonographic liver and spleen volumes were expressed as multiple of normal value, calculated for each subject function of their weight, using the accepted cut-off values of 2.5% of their body weight for the liver respectively of 0.2% of their body weight for the spleen (11, 12). The diagnosis was specifically determined for each

subject using an enzymatic and genetic assessment. The enzymatic activity of α-L-iduronidase was evaluated using the analyse of this activity in plasma (Biochemistry Department of „Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca) or the fluorometric determination of 4-methylumbelliferone using dried blood spot (Centogene Laboratory Rostock, Hamburg). These two different methods were done according to the possibilities that we had at different periods of time. Genetic testing was done respecting the ethical standards of the hospital committee, according to Helsinki Declaration of 1975 (revised in 2000). Genetic evaluation was performed at Archimed Life Science GnbH Laboratory, Vienna and Centogene Laboratory, Rostock. The genetic analysis of IDUA gene consisted in sequencing of all exons and intronic regions using NM_000203.3. Novel as reference sequence for IDUA gene. Polyphen-2 platform was used to predict the pathogenic effect of unreported mutations.

Results

The seven patients originated from 4 unrelated families, three patients/2 families (F3, F4) with severe, Hurler syndrome, two patients (F1) with intermediate, Hurler-Scheie syndrome and two with attenuated (F2) clinical phenotype, Scheie syndrome. The specific diagnosis in all 3 families with two affected children was established only after the birth of the second child, although the clinical picture in the first child was strongly suggestive for this disease.

The clinical features of the seven patients with MPS I are presented in Table I.

The height presented important variations, between -0.6 SD and -7.8 SD, depending on age, therefore the mean height was determined in two groups: in patients under 7 years, which presented -2.42 SD, respectively -6.25 SD mean height in patients aged between 7 and 17.9 years. The height was normal in the first years, then the growth velocity progressively decreases with age. In each patient was observed the classical picture of MPS type 1 syndrome: coarse facial features, clinical signs of articular and skeletal involvement with stiff joints, umbilical and/or inguinal hernia and hepatosplenomegaly. The mean volume of the liver was 2.03±1.57 multiple of normal liver volume (calculated for each subject function of his weight) and the mean spleen volume was 3.65±1.78 multiple of normal spleen volume (calculated for each subject function of his weight). Concerning the cardiac involvement, it was represented by variable degrees of left ventricular hypertrophy and valvopathies. All the patients presented mitral valve thickening with different degrees of regurgitation, depending on the age at diagnosis. Aortic insufficiency was observed in four patients. Corneal opacities in variable degree were present in five patients. Neurological and psychological assessment revealed 2 siblings with normal intellectual development and 5 patients with a variable neuro-intellectual disability, from mild deficit to severe. The mean age of clinical onset was 1.17±1.94 years; the clinical diagnosis was established at the age of 6.62±7.26 years, and specific genetic diagnosis was established at 7.74±7.12 years. It was observed an important delay (3.4± 2.36 years) between the age at clinical onset to the moment of specific genetic diagnosis (the limits: 1 month and 12.7 years). A Scheie syndrome patient (the younger sister, F3) late diagnosed, died shortly after confirmation of the diagnosis prior to initiation of enzyme replacement therapy, due to severe heart disease. The enzyme replacement therapy was started at mean the age of 6.92± 9.09 years (limits: 1.75–19 years), the average duration of therapy was 6.37 ± 5.37 years with a range between 0.25 and 13 years. The α-L-iduronidase activity presented a markedly reduction or absence in all patients (Table II.).

Table I. MPS I patients clinical characteristics

No	gender	Age: onset	Age: unspecific diagnosis	Age: specific diagnosis	Age: starting therapy	Age: current	SDS for height	Hernia	Liver volume (x N)	Splenic volume (x N)	Valvulopathy: MI	Valvulopathy: MS	Valvulopathy: AI	Valvulopathy: AS	Cardio-myopathy	Respira- tory difficulties	Osteo-arthropathy	Kerato-pathy	Neuro-logical involvement
F1-P1	F	1	2	9.5	10.25	22	-7.8	+	1.45	3.8	+	+	+	-	-	+++	+++	++	+
F1-P2	M	1.5	4.5	4.6	5.16	18	-5.88	+	1.2	2.1	+	+	+	-	-	++	++	++	-/+
F2-P1	F	5	17.75	17.9	19	24	-5.08	+	5.45	7.27	+++	-	++	-	+	+	+	-	-
F2-P2	F	5	16.5	16.75	-	Died 17													
F3-P1	F	1	2.5	2.1	2.66	6.66	-3.7	+	1.26	1.88	+	+	+	-	+	++	++	+	++
F3-P2	F	0.75	0.75	0.83	1.75	4.75	-0.6	+	1.2	3	+	+	-	-	-	++	+	+	++
F4-P1	M	1	2.4	2.5	2.75	3	-2.96	+	2.5	3.52	+	-	-	-	-	+	+	-	+

F=family; P- patient; SF= female, M-male, DS= standard deviation score; N= normal; MI=mitral valve insufficiency; MS= mitral valve stenosis; AI=aortic valve insufficiency; AS=aortic valve stenosis

The molecular analysis identified six different diseases causing pathological variants, 3 point mutations (1 nonsense and 2 missense mutations), 2 deletions, 1 insertions and 2 polymorphic sites (SNP) mutation (Table III). We found homozygosity in two siblings (F3), whereas the other five patients (F1, F2, F4) showed the presence of compound heterozygous IDUA genotype. Two of these were novel pathogenic variants.

In two sisters (F3) with severe phenotype (Hurler syndrome) was detected the c.208C>T p.Q70* homozygous mutation in exon 2 and a second homozygous mutation c.352C>T p.L118L in exon 3 - SNP.

In a F4 patient, another patient with Hurler syndrome, it was also detected the c.208C>T p.Q70* pathogenic variant in exon 2 and the second, c.46_57delTCGCTCCTG, p.S16_A19delGCC, in exon 1.

In two siblings with Hurler-Scheie syndrome (F1) two unreported mutations were identified: the c.971A>G results in a missense mutation p.K324R which is predicted to be probably damaging with a score of 1.000 by Polyhen-2 and an insertion of one base pair in exon 9 resulting in frameshift and premature stop.

In F2 family with Scheie syndrome two mutations were detected: c.713T>A missens mutation p.L238Q and a deletion c.1045_1047delGAC.

The most frequent mutation in our group was the p.Q70*.

Discussion

In this study we described the clinical and molecular characterization of the MPS type I patients, being the first one on Romanian patients. The seven patients originated from 4 unrelated families, three patients/2 families (F3, F4) with severe, two patients (F1) with

Table II. α-L-Iduronase activity

No.	Enzyme activity	Normal range	Unit of measure	Sample type
F1-P1	0	300-800	mMol/l/4h	Plasma
F1-P2	0	300-800	mMol/l/4h	Plasma
F2-P1	0	300-800	mMol/l/4h	Plasma
F2-P2	0	300-800	mMol/l/4h	Plasma
F3-P1	<0.5	≥ 2	µmol/l/h	DBS
F3-P2	<0.5	≥ 2	µmol/l/h	DBS
F4-P1	<0..5	≥ 2	µmol/l/h	DBS

F= Family; P=Patient; DBS= dried blood spot

Table III. MPS I patients molecular characteristics

Nr.	Allele	Location	Nucleotide change	Amino acid change	Reference	Biochemical phenotype	Clinical form
F1	1	Exon 8	c.971A>G	p.K324R	-	no enzyme activity	H-S
	2	Exon 9	c.1389 ins C	-	-		
F2	1	Exon 7	c.713T>A	p.L238Q	Yogalinga, 2004	no enzyme activity	S
	2	Exon 8	c.1045_1047delGAC		Bertola, 2011		
F3	1	Exon 2	c.208C>T	**p.Q70***	Clarke, 1993	low enzyme activity	H
		Exon 3	c.352C>T	p.L118L	rs37559954		
	2	Exon 2	c.208C>T	**p.Q70***	Clarke, 1993		
		Exon 3	c.352C>T	p.L118L	rs37559954		
F4	1	Exon 2	c.208C>T	p.Q70*	Clarke 1993	low enzyme activity	H
	2	Exon 1	c.46_57delTC-GCTCCTGGCC	p.S16_A19del	Bunge,1993		

F=family; SNP= single nucleotide polymorphism; H-Hurler syndrome; S=Scheie syndrome; H-S=Hurler=Scheie syndrome

intermediate and two with attenuated (F2) clinical phenotype. Each patient presented the classical picture of MPS type I picture, represented by: variable coarse facial features, arthropathy, hepatosplenomegaly, cardiac involvement, respiratory dysfunction and neurological impairment.

The p.Q70*(c.208C>T) variant was identified in 2 families with severe form of disease (Hurler syndrome) in homozygous status in one family (F3) and in compound heterozygous status in the other family (F4). The p.Q70*(c.208C>T) variant induce a stop codon, predicted to prematurely end the transcript (9). Biochemical assays already described for these variants showed essentially no activity in homozygotes (13), concordant with Hurler phenotype, in our patients being observed a very low activity for the enzyme, the clinical phenotype being also Hurler syndrome.

The incidence of p.Q70* (c.208C>T) mutation, 3/8 alleles of pathological variants of IDUA gene alleles identified in our group is according to results reported by other studies (5, 14-19).

In F4, compound heterozygous p.Q70*(c.208C>T), the second variant is a deletion located on exon 1 (p.S16_A19del, 46_57delTCGCTCCTGGCC), described by Bunge in 1994, as a mutation that induces a severe phenotype (3, 20). The phenotype for this patient, as for the siblings of F3 family, homozygous for p.Q70* (c.208C>T) was also severe, as Hurler syndrome.

In two siblings (F1) with intermediate form, Hunter-Scheie syndrome, two new mutations were identified: c.971A>G (p.K324R), which leads to a missense variant (undescribed variant at the moment of determination, in 2015, but subsequently described by Uttarilli et al in 2016 in a patient from India, with low enzyme activity) (21); and an insertion of one base pair in exon 9 resulting in frameshift and premature stop (c.1389 ins C) (not described until now). The variable expression of the different variants of IDUA gene is indicated by the large spectrum of biochemical and clinical signs (22).

In F2, two mutations were detected: c.713T>A results in a known missense mutation p.L238Q, which described reduced IDUA protein and activity level, as in our patient (23) and a deletion of 3 base pair in exon 10 (c.1045_1047delGAC), also associated with low enzymatic level (8). The patient from F2 family presented the less severe MPS I phenotype, Scheie syndrome.

Overall, most mutations were "private", with only one mutation (Q70*) being common in our group, according to other studies (8, 24).

Our results confirm the degree of variants heterogeneity observed in MPS I, in concordance with other previous studies (25, 26). This allelic heterogeneity does not permit to establish clear correlations between mutant genotypes and phenotypes (27). A limitation for our study is the limited number of patients to allow clear conclusions regarding the phenotype genotype correlations in our patients.

Conclusions

The p.Q70* missense variant was the most frequent, correlated in all the cases who presented it with severe form, Hurler syndrome, the other mutations being usually isolated and particular for each patient, associated in our patients with less severe MPS I phenotype, as Hurler-Scheie or Scheie syndrome. The results of this study indicated the mutational heterogeneity of the IDUA gene and the difficulty to indicate some correlation between the genotype and phenotype in MPS I patients.

Abbreviations

MPS I - Mucopolysaccharidosis type I
IDUA - α-L-iduronidase
GAGs - glycosaminoglycans
H - Hurler syndrome
H-S - Hurler–Scheie syndrome
S – Scheie syndrome
F - family
SF - female
M - male
DS - standard deviation score
N - normal
MI - mitral valve insufficiency
MS - mitral valve stenosis
AI - aortic valve insufficiency
AS - aortic valve stenosis
DBS - dried blood spot
SNP - single nucleotide polymorphism.

Authors' contribution

CAl (conceptualization, methodology, validation, investigation, manuscript writing, manuscript supervising)
CL (methodology, validation, investigation)
DM (methodology, manuscript writing)
CAs (methodology, validation, investigation)
IN (methodology, validation, investigation)
TP (methodology, validation, investigation)
PGS (conceptualization, methodology, validation, investigation)

Conflict of interest

None to declare.

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