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Mucopolysaccharidosis IVA: Correlation between genotype, phenotype and keratan sulfate levels

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ABSTRACT

Mucopolysaccharidosis IVA (MPS IVA) is caused by deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS), leading to systemic skeletal dysplasia because of excessive storage of keratan sulfate (KS) in chondrocytes. In an effort to determine a precise prognosis and personalized treatment, we aim to characterize clinical, biochemical, and molecular findings in MPS IVA patients, and to seek correlations between genotype, phenotype, and blood and urine KS levels. Mutation screening of *GALNS* gene was performed in 55 MPS IVA patients (severe: 36, attenuated: 13, undefined: 6) by genomic PCR followed by direct sequence analysis. Plasma and urine KS levels were measured by ELISA method. Genotype/phenotype/KS correlations were assessed when data were available. Fifty-three different mutations including 19 novel ones (41 missense, 2 nonsense, 4 small deletions, 1 insertion, and 5 splice-site) were identified in 55 patients and accounted for 93.6% of the analyzed mutant alleles. Thirty-nine mutations were associated with a severe phenotype and ten mutations with an attenuated one. Blood and urine KS concentrations in MPS IVA patients were age-dependent and markedly higher than those in age-matched normal controls. Plasma and urine KS levels in MPS IVA patients with the severe phenotype were higher than in those with an attenuated form.
This study provides evidence for extensive allelic heterogeneity of MPS IVA. Accumulation of mutations as well as clinical descriptions and KS levels allows us to predict clinical severity more precisely and should be used for evaluation of responses to potential treatment options.

1. Introduction

Mucopolysaccharidosis IVA (MPS IVA, Morquio A disease; OMIM# 253000) is an autosomal recessive lysosomal storage disorder (LSD) characterized by a loss of activity of the N-acetylgalactosamine 6-sulfate sulfatase (GALNS) enzyme. The estimated incidence of Morquio A disease varies widely between 1 in 75,000 to 500,000 births [1–8].

Deficiency of GALNS results in a build-up of the glycosaminoglycans (GAGs), keratan sulfate (KS), and chondroitin-6-sulfate (C6S) in lysosomes throughout the body, but specifically in the cartilage and cornea, where KS is synthesized. In MPS IVA, the degradation of KS is defective. KS is predominantly found in cartilage and cornea, the major organs affected in MPS IVA. The specific mechanism, by which excess storage of KS results in the skeletal dysplasia unique to MPS IVA, remains unknown.

The most widespread pathological findings are related to a systemic skeletal dysplasia including short trunk dwarfism, kyphoscoliosis, platyspondyly, odontoid hypoplasia, genu valgum, pectus carinatum, and dental anomalies. Other findings include characteristic ligamentous laxity, corneal clouding, coarse facies, hearing loss, and valvular heart disease. Unlike other MPS disorders, there is no central nervous system involvement and intelligence is preserved [9]. There is variable severity, but patients with severe phenotype usually do not survive past the second or third decade of life. Patients with the attenuated form of MPS IVA have been reported to survive into the seventh decade of life [10]. Based

Abbreviations: C6S, Chondroitin-6-sulfate; CDC, Centers for Disease Control and Prevention; Cr, Creatinine; DMB, dimethylmethylene blue; DMSO, dimethylsulfoxide; GAGs, Glycosaminoglycans; GALNS, N-acetylgalactosamine-6-sulfate sufatase; IMO, International Morquio Organization; KS, Keratan sulfate; LSD, Lysosomal storage disorder; MPS, IVA Mucopolysaccharidosis IVA.

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on a natural history study by the International Registry program [9], around 50% of the subjects underwent orthopedic surgical procedures. The patients with more severe short stature and those who underwent surgical procedures were reported to have more difficulties ambulating. The current clinical criteria establish that reduced growth and final height are associated with a more severe clinical phenotype.

The GALNS gene, located on chromosome 16q24.3, contains 14 exons spanning 50 kb and encodes a 522-amino acid protein, including a signal peptide of 26 residues [11,12]. GALNS has been purified from human placenta as an oligomer of 40 and 15 kDa polypeptides [13], with the oligomers inter-linked by disulphide bonding. Mature human GALNS enzyme is stabilized in a complex with two other

Table 1
Characterization of MPS IVA patients in this study.

MO	Sex	Phenotype	Age tested for height and weight (years)	Present height (cm)	Percentile in Morquio patients	Present weight (kg)	Percentile in Morquio patients	Age onset	Age diagnosis	Growth arrest	Orthopedic Surgery	1st allele: Nucleotide change	2nd allele: Nucleotide change	Ethnicity
MO 58	M	undefined	8.0	NA		NA		NA	NA	NA	NA	c.1023C>G	undefined	SL
MO 59	F	undefined	6.0	NA		NA		NA	NA	NA	NA	c.1023C>G	undefined	SL
MO 100	M	severe	9.9	98.4	≦25th	15.9	≦10th	0.5	0.6	no	+	c.853_855delTTC	undefined	Am–Ca (Br/Ge/Pt)
MO 101	M	attenuated	12.0	127	≧75th	38.6	≧90th	5	5	no	–	c.758G>A	c.922T>C	Am–Ca (Al)
MO 104	M	attenuated	24.0	127	≧75th	52.5	≧75th	1	2	yes	–	c.121A>T	c.121A>T	Am–Ca (Fr)
MO 105	F	severe	13.0	102	≦50th	21	≦50th	0.5	6	yes	+	c.898+1G>A	c.121–1G>C	Uk
MO 106	M	attenuated	13.0	150	≧90th	45	≧90th	5.5	6.4	no	–	c.320–1G>T	undefined	Chil
MO 107	F	attenuated	18.0	120	≧75th	31.8	≦75th	4	4.5	yes	+	c.1354T>A	c.1485C>G	Ca–Ca (Ir/En)
MO 109	M	severe	13.0	116	≦75th	24.5	≦50th	1	3	yes	+	c.415G>A	c.1219A>C	Am–Bl, Am–Ca
MO 110	M	severe	12.5	105	≦50th	19.85	≦25th	NA	3.8	yes	–	c.715G>T	c.715G>T	Br
MO 112	M	severe	7.0	94.5	≦25th	20	≦75th	NA	5.7	no	–	c.901G>T	c.901G>T	Br
MO 113	M	severe	7.4	100	≦50th	17.6	≦50th	1	NA	no	+	c.1023C>G	c.1156C>T	Br
MO 114	M	severe	11.6	95	≦25th	16	≦10th	NA	NA	yes	–	c.1023C>G	c.1023C>G	Br
MO 115	F	severe	11.5	90	≦10th	13	≦10th	NA	3.3	yes	–	c.280C>T	c.608C>T	Br
MO 116	M	severe	12.4	109	≦75th	21.3	≦25th	NA	7.1	yes	–	c.280C>T	c.608C>T	Br
MO 117	F	severe	8.3	91.4	≦25th	13.6	≦10th	1.3	2	no	+	c.346G>A	c.1156C>T	Am–Ca
MO 117b	F	severe	6.8	86.4	≦10th	14.1	≦25th	NA	3.5	no	–	c.346G>A	c.1156C>T	Am–Ca (It)
MO 120	F	undefined	4.5	NA		NA		NA	NA	NA	NA	c.612C>G	c.612C>G	Am–Ca
MO 121	M	attenuated	16.0	150	≧90th	63.4	≧90th	9	14	yes	+	c.181C>T	c.498delC	Am–Ca
MO 121s	F	attenuated	11.0	142.8	≧90th			8	8	yes	–	c.181C>T	c.498delC	Am–Ca
MO 122	M	attenuated	9.0	130	≧90th	35	≧90th	NA	NA	no	–	c.975G>T	c.1156C>T	Ca–Ca
MO 125	M	attenuated	7.2	124	≧90th	26.4	≧90th	3	6	no	+	c.29G>A	c.G1215>A	Au
MO 126	M	severe	9.0	104.1	≦50th	17.7	≦25th	at birth	1.4	yes	–	c.278T>A	undefined	Am–Ca
MO 127	M	severe	14.7	102	≦50th	20	≦25th	0.5	9	yes	+	c.3G > A	c.257T>C	Fi
MO 129	F	undefined	0.8	NA		NA		NA	NA	NA	–	c.634–1 G>T	c.860C>T	Gr
MO 133	M	severe	24.0	97	≦25th	28	≦50th	0.65	3	yes	+	c.139G>A	c.139G>A	Po
MO 134	M	severe	2.7	85	≦25th	10	≦10th	0.6	1.7	yes	–	c.697G>A	undefined	Po
MO 138	F	severe	12.7	NA		NA		NA	NA	NA	–	c.477G>A	c.477G>A	Am–Ca
MO 139	F	severe	11.0	94	≦25th	17	≦25th	1.5	1.5	yes	+	c.230C>G	c.230C>G	SA
MO 141	M	undefined	33.0	NA		NA		NA	NA	NA	NA	c.1171A>G	undefined	Ca–Ca
MO 143	M	severe	4.0	93.6	≦50th	16	≦50th	2	4.5	no	–	c.860C>T	c.860C>T	Mac
MO 144	F	severe	23.3	101.6	≦50th	22.7	≦25th	4	4	yes	+	c.953T>G	c.1567T>G	Ch
MO 145	M	severe	12.0	113	≦75th	20	≦25th	NA	3	yes	+	c.1156C>T	c.1219A>C	Fr
MO 146	F	severe	11.0	111.8	≦75th	20.86	≦50th	2	6.5	yes	–	c.1171A>G	c.1171A>G	Am–Ca (En/Ge)
MO 147	F	severe	4.1	91.4	≦75th	13.2	≦50th	at birth	0.5	no	+	c.901G>T	c.901G>T	Am–Ca
MO 148	M	severe	8.0	107	≦50th	22	≦75th	2.2	4.3	yes	–	c.422+2_+3insT	c.1195delA	Ira
MO 148s	F	severe	5.3	103	≦50th	18	≧90th	1.5	1.7	yes	–	c.422+2_+3insT	c.1195delA	Ira
MO 150	F	undefined	6.9	NA		NA		NA	NA	NA	NA	c.485C>T	c.485C>T	Co
MO 151	M	severe	4.0	91.44	≦50th	13.6	≦50th	1.5	2.5	no	+	c.121–1G>A	c.337A>T	Am–Ca
MO 154	F	severe	1.8	81	≦50th	11.5	≦25th	0.5	1.5	no	+	c.1156C>T	c.1156C>T	Am–Ca (His/Gr)
MO 155	M	severe	14.4	94.3	≦25th	18.6	≦25th	2.5	2.9	yes	–	c.452C>T	c.452C>T	Pa
MO 157	M	severe	5.5	89.2	≦25th	NA		NA	NA	NA	NA	c.938C>T	c.938C>T	Ca–Ca
MO 158	F	severe	3.8	88.9	≦75th	12.08	≦50th	2.3	2.6	no	+	c.740G>A	c.901G>T	Am–Ca (Ge/Sw)
MO 159	F	severe	3.9	90	≦75th	13.2	≦50th	1	1.5	no	+	c.346G>A	c.860C>T	Tu
MO 160	F	severe	4.0	91.4	≦75th	12.7	≦50th	0.7	1.3	no	+	c.346G>A	c.1485C>G	Am–Ca
MO 161	M	severe	18.0	117	≦75th	33	≦50th	2	1.6	yes	+	c.125G>A	c.374C>T	It
MO 162	M	severe	8.2	101	≦50th	16	≦25th	1.5	2	yes	–	c.415G>A	c.901G>T	Sp
MO 163	M	attenuated	9.3	139.7	≧90th	34.1	≧90th	2.5	3.8	no	+	c.1171A>G	c.1354T>A	Ca–Ca
MO 163s	F	attenuated	5.0	116.8	≧90th	25	≧90th	2.3	0.8	no	–	c.1171A>G	c.1354T>A	Ca–Ca
MO 165	M	severe	7.0	81.3	≦10th	10.9	≦10th	0.8	1.7	no	–	c.405_422+1del19	c.1480A>G	Ca–Ca
MO 166	F	attenuated	10.2	126.6	≧90th	NA		NA	NA	no	NA	c.244T>C	c.244T>C	Ca
MO 167	F	attenuated	46.9	139.7	≧75th	47.2	≧75th	7	5	yes	–	c.740G>A	c.761A>G	Am–Ca
MO 168	F	attenuated	10.1	114	≧75th	22.2	≦75th	2	6.5	no	–	c.850T>G	c.850T>G	Tu
MO 170	F	severe	27.0	91.4	≦25th	22.7	≦25th	2	3	yes	+	c.122T>A	c.122T>A	Am–Ca
MO 172	M	severe	18.8	91.4	≦10th	19.07	≦10th	2	3.2	yes	+	c.245C>T	c.498delC	Am–Ca

*The DNA mutation numbering is based on cDNA sequence. Nucleotides numbered from the ATG initiator codons.
#Al: Albanian, Am-Ca: American Caucasian, Am-Bl: American-Black, Ar: Argentine, Au: Austrian, Br: Brazilian, Bt: British, Ca-Ca: Canadian Caucasian, Ch: Chinese, Chil: Chilean, Co: Colombian Fi: Finnish, Fr: French, Ge: German, Gr: Greek, Hi: Hispanic, It: Italian, Ira: Iraq, Ir: Irish, Jp: Japanese, Mac: Macedonian, Pk: Pakistani, Po: Polish, Pt: Portuguese, SA: Saudi Arabian, Sw: Swedish, SL: Sri Lanka, Sp: Spanish, Tu: Turkish, un: unknown, Uk: Ukrainian.
Highlighted in gray: novel mutations.