

DIAGNOSIS METHOD FOR MUCOPOLYSACCHARIDOSES

by

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“Education is the key to unlocking the world, a passport to freedom.”

-Oprah Winfrey

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LIST OF ABBREVIATIONS

3-MSCT- 3-Minute Stair Climb Test
4MU-Gal6S- 4-methylumbelliferyl- β -D-galactopyranoside-6-sulfate
6-MWT- 6-Minute Walk Test
ABR-Auditory Brainstem Response
ADL- Activity of daily living
ARSB- Arylsulfatase B
BAL- Broncho alveolar lavage
BBB-Blood brain barrier
BMD- Bone Mineral Density
C6S- Chondroitin-6-sulfate
CDC- Centers for Disease Control and Prevention
CNS- Central nervous system
CS- Chondroitin sulfate
CSF-Cerebrospinal Fluid
CT-Computed Tomography
DBS- Dried Blood Spot
DMC- Dyggve-Melchior-Clausen syndrome
DMMB- Dimethylmethylene Blue
DS- Dermatan Sulfate
DXA- Dual Energy X-Ray Absorptiometry
ECG- Electrocardiography
ECM- Extracellular Matrix
EGF- Epidermal growth factor
ELISA- Enzyme-linked immunosorbent assay
ERT- Enzyme replacement therapy
FDT- Functional Dexterity Test
FET- Forced Expiratory Time
FEV₁- Forced Expiratory Volume in 1 Second
FIVC- Forced Inspiratory Vital Capacity
FVC- Forced Vital Capacity
G6S- Galactose-6-sulfatase
GAGs- Glycosaminoglycan
Gal- D-galactose
Gal-6S- Galactose-6-Sulfate
GalNAc-6S- N-acetylgalactosamine-6-sulfate
GALNS- N-acetylgalactosamine-6-sulfate sulfatase
GLB1- B-galactosidase
GlcNAc- N-acetyl-D-glucosamine
GLcNS- N-sulfoglucosamine

GM1-Gangliosidosis
GNS- N-acetyl-glucosamine 6-sulfatase
GUSB- β -D-glucuronidase
HGSNAT- α -glucosaminidase acetyltransferase
HS- Heparan sulfate
HSCT- Hematopoietic stem cell transplantation
I2S- Iduronate-2-sulfatase
IDUA- α -L-iduronidase
IEMs- Inborn errors of metabolism
IL-1b- Intleukin-1beta
IOC-Impulse Oscillation
ITT- Intent-to-Treat
KS- Keratan sulfate
LC- Liquid chromatography
LC-MS/MS- Liquid chromatography tandem mass spectrometry
LDF- Lateral Distal Femur
LS- Lumbar Spine
LSD- Lysosomal storage disorders
M6P- Mannose-6-phosphate
M6PR- Mannose-6-phosphate receptor
MIP-1a- Macrophage inflammatory protein 1 alpha
MPS- Mucopolysaccharidoses
MPS IVA- Morquio A Syndrome
MRI- Magnetic Resonance Imaging
MS/MS- Tandem mass spectrometry
MVV- Maximum Voluntary Ventilation
NAGLU- α -N-acetylglucosaminidase
NBS- Newborn screening
NO- Nitric oxide
PFT- Pulmonary Function Tests
PGs- Proteoglycans
PIIANP- Type IIA Collagen N propeptide
QF-PCRs- Quantitative Fluorescent-PCRs
RBC- Red blood cells
ROM- Range of Motion
SDB- Sleep Disordered Breathing
SGHS- Heparan-N-sulfatase
SNS- Somatic nervous system
SRT- Substrate reduction therapy
TLC- Total Lung Capacity
TLC- Thin-layer chromatography
TNF- α - Tumor necrosis factor-alpha
VC- Vital Capacity

VRA- Visual Reinforcement Audiometry
WB- Whole Body
WHO-World Health Organization

ABSTRACT

Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases caused by the deficiency of lysosomal enzyme that is required to degrade various glycosaminoglycans (GAGs). GAGs are long unbranched polysaccharides consisting of repeating disaccharides that include chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, and hyaluronan. Each type of MPS is characterized by the accumulation of specific GAG(s). In MPS, the undegraded GAGs are stored in lysosomes and extracellular matrix (ECM) of a variety of tissues, secreted into the bloodstream, and then excreted in the urine. Accumulated GAGs lead to cell dysfunction and abnormal structure of ECM, causing progressive damage of multiple tissues including CNS, lung, heart, liver, spleen, kidney, joint, and bone. There are 11 known enzyme deficiencies, resulting in seven distinct forms of MPS.

The first aim of this study was to obtain data about the epidemiology of the different types of MPS in Japan and Switzerland and to compare with similar data from other countries.

Overall, the frequency of MPS varies for each population due to differences in ethnic backgrounds and/or founder effects that affect the birth prevalence of each type of MPS, as seen for other rare genetic diseases.

The second aim was to explore clinical, radiographic, biochemical, and molecular diagnosis and clinical assessment tests for Mucopolysaccharidosis IVA (MPS IVA, Morquio A syndrome).

MPS IVA is one of MPS disorders inherited as an autosomal recessive trait and caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase. Deficiency of this enzyme leads to the accumulation of specific glycosaminoglycans (GAGs), chondroitin-6-sulfate (C6S) and keratan sulfate (KS), which are mainly synthesized in the cartilage., leading to a direct impact on bone development and successive systemic skeletal spondylepiphyseal dysplasia. Diagnosis of MPS IVA needs clinical, radiographic, and laboratory testing to make a complete conclusion. After investigation, urinary and blood KS and C6S, the enzyme activity of GALNS, and *GALNS* molecular analysis are used for diagnosis and prognosis of clinical phenotype in MPS IVA.

The third aim was to determine the appropriate biomarker for screening MPS and monitoring therapeutic efficacy. We identified significant increase of pro-inflammatory factors such as cytokines and chemokines and GAGs in MPS IVB, MPS IVA, and MPS II patients.

Overall accurate and rapid diagnosis for MPS is required to manage and treat the patients with MPS.

Chapter 1

INTRODUCTION

1.1 Lysosomal Storage Disorders

The lysosome was first discovered in 1955 by De Duve et al. [1]. Lysosomes are organelles found in all mammalian cells except red blood cells (RBC) and are part of a highly dynamic endocytotic system while receiving input from the biosynthetic pathways as well [2]. There are several hundred lysosomes in each mammalian cells which are heterogeneous in morphology and size, about 100-1,000 nm, which constitutes about 5% of the cell volume [3]. The biggest function of lysosomes is part of the digestive system, where they degrade materials taken up from outside of the cell as well as to digest dying components of the cell itself [4]. The common function of lysosomes is also known as destroying intracellular material [4].

Lysosomes are acidic, hydrolase-rich organelles that are capable of degrading most biological macromolecules [2]. Lysosomes have more than 50 different degradative enzymes which can hydrolyze several important substrates in the cell [4]. Lysosomes are responsible for the breakdown of various biomaterials and macromolecules, such as membranes, proteins, polysaccharides, and complex lipids into their respective smaller building-block molecules: amino acids, monosaccharides, free fatty acids, or nucleotides [5–8]. Lysosomes have two main functions; which are degradation and signaling functions, causing lysosomes to partake in a broad range of cellular and physiological functions such as secretion, plasma membrane repair, cell

growth, cholesterol homeostasis, autophagy, bone and tissue remodeling, pathogen defense, cell signaling and death [2,6].

These lysosomal enzymes are translated in the endoplasmic reticulum (ER) and then transported from the Golgi Apparatus to the lysosome via a mannose-6-phosphate receptor (M6PR) dependent mechanism [9]. Acid hydrolyses are targeted to lysosomes by mannose-6-phosphate (M6P) residues, which are recognized by M6PR in the trans Golgi network [4]. Lysosomal enzymes main function is to break down complex sugars, lipids, glycolipids, GAGs, nucleic acids, and proteins [9]. All lysosomal enzymes are an acid hydrolysis, which are functioning at an acidic pH level of 4 to 5 within the lysosomes [4,9]. To maintain the acidic internal pH, lysosomes must actively concentrate protons, H⁺ ions, via a proton pump in the lysosomal membrane [4]. The low pH of the endosomal compartments causes the lysosomal enzyme to dissociate of the endosomal compartment which are then delivered to lysosomes, whereas the M6P receptors cycle back to the trans-Golgi network to further transport [2]. The acidification of endosomes, lysosomes and even lysosome-related organelles facilitate the dissociation of the M6P-receptor-ligand complexes, and the proteolytic processing required for the enzymatic activation of several hydrolases [2].

Lysosomal formation is a combination between where the lysosomal proteins are processed, the secretary pathway, and the endocytic pathway, where the extraocular molecules are taken up at the cell surface [4]. Complex lipids are usually delivered to lysosomes for degradation as parts of the auto phagocytosed or endocytosed membranes [6], which send outputs through retrograde trafficking and lysosomal exocytosis [10]. Misregulation of the balance between endocytosis and

exocytosis can result in accumulation of undigested materials and dysfunctional lysosomes [10]. With the help of specific chaperons, lysosomal membrane proteins can help the selective transport of cytosolic substrates into the lysosome [2].

The diversion of the lysosomal enzymes from the secretory pathway is dependent on the acquisition of the M6P recognition marker [11,12]. The binding of ligands to the extracellular domain of the cation-dependent M6P receptor is pH dependent [2]. To protect the perimeter membrane of the lysosome and its membrane proteins from degradation, the inner leaflet is coated with a polysaccharide layer known as the glycocalyx [13].

Lysosomal storage disorders (LSD) are due to congenital inborn errors of metabolism, which results in the absence of the deficiency of an enzyme, leading to an inappropriate storage of material in various cells of the body [14]. Accumulation of undigested sugars, lipids or other biomaterials in the lysosome lead to LSDs [15].

LSDs are usually caused by the lack of hydrolase, its activator or a transporter causing accumulation of specific substrates in the lysosomes for each disorder type [16]. Most LSD are inherited through autosomal recessive manner, except for Fabry disease [17], Hunter (MPS II) disease [18], and Danon disease [19], which are all X-linked recessive.

To date, there are about 50 LSD that are characterized by an accumulation of waste products in the lysosomes [16]. The severity of a LSD disease depends on the type of accumulating waste product, which cells or tissues accumulate waste products, genetic background and environmental factors [16]. Most patients with LSD are born apparently healthy, as the symptoms progressively develop [16]. The age of onset, severity of symptoms and the central nervous system (CNS) manifestation can vary

impressively within each single disorder type because LSDs have a broad spectrum of clinical phenotypes. The severity of these LSDs may correspond to the degree of residual enzyme activity, giving rise to phenotypes ranging from severe early-onset forms to milder late-onset forms [2]. Many LSDs develop progressively with age, and several involve the CNS are associated with mental retardation [2]. Even a small increase in the residual enzyme activity could significantly impact the disease development [20].

The incidence of LSDs as a group is estimated to be 1:7,000 to 1:8,000 [21]. Although the total number of lysosomes is not reduced in LSDs, the overall function of the lysosomes in a cell is compromised, leading to severe cellular consequences [10].

1.2 Mucopolysaccharidosis (MPS)

Mucopolysaccharidoses (MPS) are a group of LSDs caused by the deficiency of enzymes required to degrade glycosaminoglycans (GAGs) in the lysosome. GAGs are sulfated polysaccharides comprising of repeated disaccharide of chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), keratan sulfate (KS), and hyaluronan. Hyaluronan is an exception in the GAG family because it is a non-sulfated polysaccharide [22]. Lysosomal enzymes are crucial for stepwise degradation of GAGs to provide a normal function of tissues and extracellular matrix (ECM).

The deficiency of one or more lysosomal enzyme(s) results in an accumulation of undegraded GAGs which leads to progressive tissue damage in multiple organs causing disease-specific manifestations (e.g. coarse facial features, skeletal dysplasia, hepatosplenomegaly, corneal clouding, joint rigidity or laxity, cardiac and respiratory complications, and neurological impairment) [23]. The accumulated GAGs in various

tissues and their ECM are secreted into the blood circulation and then excreted in urine. The MPS are classified according to the deficient enzyme as well as the undegraded GAGs and comprise 11 distinct subtypes, with an estimated combined incidence of 1 in 25,000 live births [24].

The primary defect in MPS leads to GAG storage, however the secondary accumulation of ganglioside in the brains is a factor for neuropathology for patients affected with MPS [25,26]. Gangliosides are sialic acid-containing glycosphingolipids that are most abundant in the nervous system [27]. Gangliosides are primarily localized in the outer leaflets of plasma membrane and are involved in cell-cell recognition and adhesion and signal transduction within specific cell surface microdomains, termed caveolae [28], lipid rafts [29] or glycosphingolipid [27,30]. The transport of neuronal growth factors is important for neuronal survival and function. Alterations in these vesicular traffic have been implicated in MPS neuropathology [31–33].

Cellular and foreign material destined for degradation reach lysosomes via endocytosis, phagocytosis, autophagy, or direct transport [34]. Brain pathogenesis in MPS patients is also due to impaired autophagy [35]. Impaired autophagy is postulated to play a major role in disease pathogenesis [35]. The presence of activated microglia, elevated levels of inflammatory cytokines as well as oxidative stress all are causes for the inflammation of neuropathology with MPS [31,36,37]. Autophagocytosis, apoptosis, inflammation as well as impaired neurotransmission are all implicated in the devastating pathology for MPS [15].

1.3 Types of MPS

There are seven types of MPS that are categorized based on either the lack or the defect in one of the eleven specific lysosomal enzymes that are described as MPS I through MPS IX. MPS V and MPS VIII are excluded, as they are no longer used. The clinical features also vary with the type of MPS and clinical severity also differs with each type of disease. MPS are autosomal recessive disorders except for MPS II that is X-linked [22]. Most clinical signs and symptoms for MPS patient do not appear immediately after birth; however, the onset of clinical signs and symptoms progress as the patient ages. The symptoms and severity of MPS vary with each patient and its specific type of MPS; however, the average life span in most patients is one to two decades, if patients are left untreated [38].

1.3.1 MPS I (Hurler, Hurler/Scheie, Scheie syndrome)

Mucopolysaccharidosis I (MPS I) is an autosomal recessive disorder [39] caused by the deficiency of α -L-iduronidase (IDUA) and leading to an accumulation of dermatan sulfate (DS) and heparan sulfate (HS). . MPS I is divided into three separate phenotypes by clinical severity: Hurler syndrome (severe), Hurler-Scheie syndrome (intermediate), and Scheie syndrome (mild). However, no feasible measurable biochemical differences have been identified [40].

The clinical findings of MPS I patients overlap as the patients have disproportionate dwarfism, joint pain, loud breathing, muscle weakness, sleep apnea, coarse hair, large head, coarse face, corneal clouding, visual impairment, dental abnormalities, large tongue, large mandible, cardiac valve abnormalities, scoliosis, kyphosis, hepatosplenomegaly, umbilical hernia, inguinal hernia, hip deformity, and genu valgum. MPS I patients have CNS impairment and developmental delay, joint

stiffness, sleep disturbance, hydrocephalus and claw hands [39,41–43]. Scheie syndrome, the milder form of the disease, is characterized by a coarse face and stocky physique with normal intelligence while Hurler syndrome displays neurological symptoms such as dyslexia, thermoanesthesia, and cognitive degradation. Patients with Hurler syndrome develop initial symptoms such as hernias, hepatomegaly, kyphosis and developmental delay within a year and die within a decade if untreated while Scheie syndrome patients can live to more than 50 years [24].

1.3.2 MPS II (Hunter Syndrome)

Mucopolysaccharidosis type II, also known as Hunter syndrome, is the only MPS form that is a X-linked lysosomal storage disorder (resulting in mostly males being affected) and is caused by mutations in the IDS gene [44]. MPS II leads to a deficiency in iduronate-2-sulfatase (I2S) enzyme and causes accumulation of DS and HS. Like MPS I, MPS II also has both, mild and severe forms; however, their clinical symptoms are different [22].

Clinical features of MPS II patients usually become apparent by two to four years of age. Patients with the mild form of MPS II reach adulthood while patients affected by the severe form usually die within the first two decades of life. The mild form is analogous to MPS I mild form, with a longer life span, a slower progression of somatic deterioration and retention of intelligence [22]. MPS II with a severe form has features similar to Hurler syndrome, except for the lack of corneal clouding and slower central (CNS) and somatic (SNS) nervous system involvement. MPS II patients, have anterior breaking of the vertebral bodies, disproportionate dwarfism, short stature, joint pain, muscle weakness, coarse hair, large head, coarse face, corneal clouding, hearing loss, dental abnormalities, hypertrophic adenoid and tonsil, large

tongue, recurrent otitis media, recurrent respiratory function, cardiac valve abnormalities, chest abnormality, cervical instability, kyphosis/gibbus, hepatosplenomegaly, umbilical hernia, inguinal hernia, and hip deformity. MPS II patients have CNS impairment, developmental delay, joint stiffness, sleep disturbance, speech delay, hydrocephalus, loud breathing, and claw hands [45–47].

1.3.3 MPS III (Sanfilippo Syndrome)

MPS III also known as Sanfilippo syndrome has four different types: MPS IIIA, IIIB, IIIC and IIID due to the lack of heparan-N-sulfatase (SGHS), α -N-acetylglucosaminidase (NAGLU), α -glucosaminidase acetyltransferase (HGSNAT) and N-acetyl-glucosamine 6-sulfatase (GNS) respectively. A defect in any of the four enzymes causes an accumulation of HS. MPS III is characterized by severe CNS degeneration and progressive developmental delay and mental retardation [22].

MPS III is clinically characterized by severe degeneration of the CNS with mild somatic disease [48]. Clinical signs and symptoms include language delay, hyperactivity, aggressive behavior, developmental delays, hirsutism and sleep disorders [49]. Anxiety, hyperactivity and aggressive behavior are major symptoms for MPS III [50]. The most severe and common form of MPS III, is type A, due to its early onset, rapid clinical course and short survival [51]. The most common initial symptoms are speech delay, dysmorphology, hearing loss and hepatomegaly [52].

Patients with MPS III initially show symptoms between two to six years of age [48]. Patients usually die at the end of the second or beginning of the third decade of life, although survival into the fourth decade has been reported [53].

1.3.4 MPS IV (Morquio Syndrome)

MPS IV also known as Morquio syndrome is caused by the deficiency of any of two distinct enzymes, N-acetylgalactosamine-6-sulfate sulfatase (GALNS) and B-galactosidase (GLB1) resulting in MPS IVA and IVB. In MPS IVA, C6S and KS accumulate in MPS IVA whereas only KS accumulates in MPS IVB. Patients with MPS IVA have normal intellectual abilities as well as less coarsening of the facial features [54]. Laxity of joints (atlanto-axial, fingers, hands, knee), atlanto-axial instability, weakness of wrist, and knock-knee are more common in patients with MPS IVA in comparison to other MPS types [54]. Vision in patients with MPS IVA is better compared to other types of MPS. Patients with a severe phenotype accounted for nearly 75% of the 399 MPS IVA patients registered in International MPS IV Registry [55]. Individuals who are diagnosed with a less severe form of the disease may still have symptoms and complications that lead to significant morbidity and disability and may be present with less severe to moderate physical disabilities [55].

With the skeletal involvement, significant morbidity of patients can also result from obstructive sleep apnea, corneal clouding, hearing impairment, respiratory compromise, valvular heart disease and spinal cord compression. Through clinical phenotypes, MPS IVB is extremely hard to distinguish from MPS IVA, because many of the characteristics overlap each other. The clinical features of MPS IVB are usually milder than those associated with MPS IVA. MPS IVA can only be set apart from MPS IVB by biochemical and/or molecular genetic testing [54].

1.3.5 MPS VI (Maroteaux-Lamy Syndrome)

Mucopolysaccharidosis type VI is also known as Maroteaux-Lamy syndrome is an autosomal recessive disorder, caused by mutations in the arylsulfatase B (ARSB)

gene and leads to a deficiency in the N-acetylgalactosamine 4-sulfatase enzyme causing an accumulation of C4S and DS [56]. The clinical presentation of MPS VI varies depending on the age of onset and rate of disease progression [57]. Some affected individuals only experience a few mild symptoms while others develop a more severe form of the disorder.

MPS VI patients have anterior breaking of the vertebral bodies, lack CNS impairment, no developmental delay, normal intelligence, disproportionate dwarfism, short stature, joint pain, sleep apnea, rigidity of joints, large head, coarse face, corneal clouding, visual impairment, hearing loss, dental abnormalities, hypertrophic adenoid and tonsil, large tongue, recurrent respiratory infection, recurrent otitis media, large mandibular, short neck, cardiac valve abnormalities, cervical instability, odontoid hypoplasia, kyphosis/gibbus, scoliosis, chest abnormalities, pectus carinatum, hepatosplenomegaly, hip deformity, genu valgum, umbilical hernia, inguinal hernia, and waddling gait [58–60].

1.3.6 MPS VII (Sly syndrome)

Mucopolysaccharidosis type VII known as Sly syndrome is an extremely rare recessive lysosomal storage disease caused by the mutations in the GUSB gene leading to a deficiency in the β -glucuronidase enzyme causing accumulation in the DS, HS, and C6S [61,62].

MPS VII patients have anterior breaking of the vertebral bodies, disproportionate dwarfism, short stature, joint pain, sleep apnea, rigidity of joints, loud breathing, snoring, muscle weakness, platyspondyly, coarse hair, large head, coarse face, corneal clouding, visual impairment, hearing loss, dental abnormalities, hypertrophic adenoid and tonsil, large tongue, recurrent respiratory infection, recurrent

otitis media, short neck, cardiac valve abnormalities, cervical instability, odontoid hypoplasia, kyphosis/gibbus, scoliosis, chest abnormalities, pectus carinatum, hepatosplenomegaly, short trunk, hip deformity, genu valgum, umbilical hernia, inguinal hernia, and waddling gait. MPS VII patients have abnormal behavior, CNS impairment, developmental delay, hyperactivity, joint stiffness, hydrocephalus, claw hands and fetal hydrops [61,63–65].

1.3.7 MPS IX (Natowicz Syndrome)

MPS IX is an extremely rare form of MPS due to the deficiency of the enzyme hyaluronidase causing an accumulation of hyaluronic acid [16]. The putatively causative mutations were found in the HYAL1 gene, encoding one of three hyaluronidases [66]. Symptoms include short stature, cysts, frequent ear infections, cleft palate and the development of soft-tissue masses [22].

1.4 MPS Age Diagnosis

Severe MPS I patients show signs and symptoms typically appearing in infancy with a median age of death of 6.8 years when untreated [67]. Patients with Scheie syndrome present later in childhood and demonstrate slower symptom progression with preservation of cognition and survival into adulthood [68]. The severe form of MPS I usually occurs before 1 year of age with skeletal deformity and coarse face followed by progressive neurological and somatic involvement [16]. The onset for the degeneration of the CNS is usually between 2 and 4 years of age,

whereas severe CNS disorder can be seen from 6 to 10 years of age, followed by early death [16]. Children with MPS II generally appear normal at birth. Young et al. noted that patients with severe MPS had an average age of onset of symptoms at around 2.5 years of age, whereas for attenuated patients the average age of symptoms was 4.3 years of age [69]. Subsequently, the average age of death for severe patients was 11.8 years and 21.7 years for attenuated patients [69].



Figure 1: Image of a patient with attenuated MPS II. L to R, patient age is 6 months, 12 months, 4 years and 5 years (adapted from Educational CD for Hunter Syndrome) [70]. Clinical features shown here are large prominent forehead, busy eyebrows, flat nose bridge, prominent cheeks/nose, large tongue, short neck and small shoulders.



Figure 2: Image of a 3-year-old patient with severe MPS II (adapted from Educational CD for Hunter Syndrome) [70]. Clinical features shown here are a coarse thick hair, large head circumference, prominent forehead, low ears/large lobes, short nose, flat nose bridge, thick tongue and thick skin.

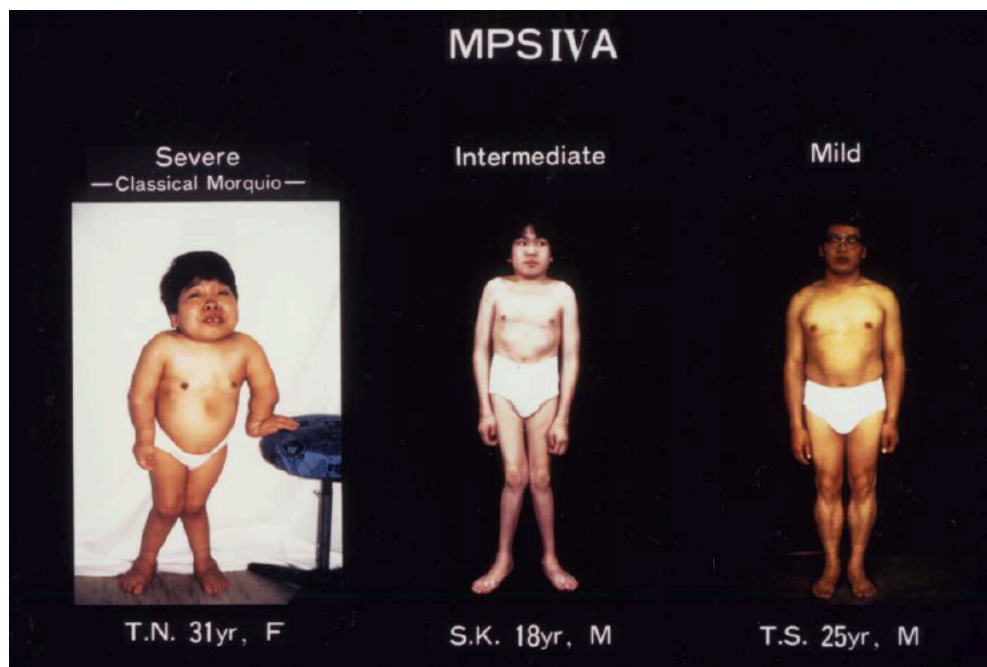


Figure 3: Images of patients with distinct differences in clinical features from mild, immediate, and severe forms of MPS IVA (adapted from Educational CD for Morquio and permitted by Carol Ann Foundation) [70].



Figure 4: Images of a patient with severe form of MPS IVA. From L to R: patient age is 7, 8, 11, 16, and 16 years (adapted from Educational CD for Morquio and permitted by Carol Ann Foundation) [70].

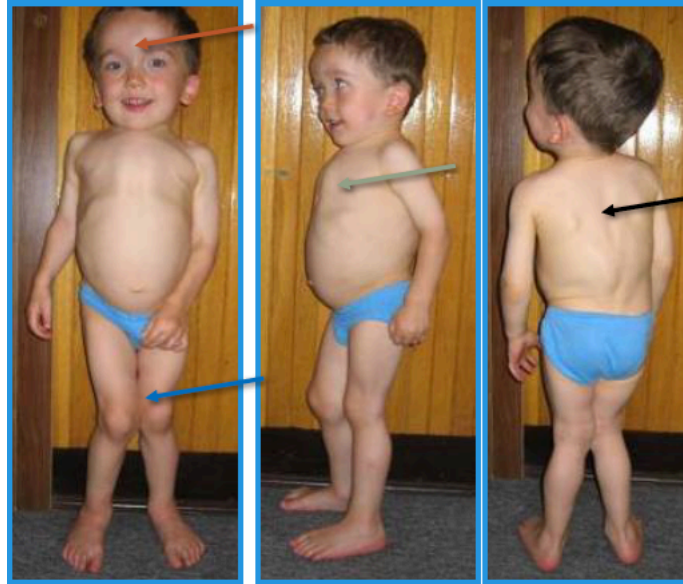


Figure 5: Image of a 3-year-old patient with severe MPS IVA. Shown here is prominent forehead (red arrow), pectus carinatum (green arrow), kyphoscoliosis (purple arrow), and genu valgum (blue arrow).

	MPS IVA	MPS IVB	MPS II
Abnormal Behavior	-	-	++
Anterior Breaking: vertebral bodies	+++	++	+++
Cardiac Valve Abnormalities	++	++	++
Cervical Instability	+++	++	++
Chest Abnormalities: rib flaring	+++	+++	++
Claw Hands (rigid fingers and hands)	-	-	+++
CNS Impairment	-	-	++
Coarse Face	+	+	++
Coarse Hair	+	+	+++
Corneal Clouding	++	++	+
Cranofacial Dysmorphia	-	-	++
Dental Abnormalities: widely spaced teeth	+++	++	+++
Developmental Delay	-	-	++
Disproportionate Dwarfism	+++	+++	++
Fetal Hydrops	-	-	-
Hearing Loss	++	++	++
Hepatosplenomegaly	+	+	++
Hip Deformity	++	++	++
Hydrocephalus	-	-	+
Hyperactivity	-	-	++
Hypermobile joint (weak wrist and double fingers)	+++	++	-
Hypertrophic adenoid and tonsil	++	++	++
Inguinal Hernia	+	+	++
Joint Pain	+++	+++	+++
Joint Stiffness	-	-	+++
Knock Knee (genu valgum)	+++	++	-
Kyphosis/Gibbus	+++	+++	+
Large Head	++	++	++
Large Mandible	++	++	-
Large Tongue	++	+	++
Loud breathing, shortness of breath	+++	+++	-
Muscular Weakness	+++	+++	+++
Normal Intelligence	+++	+++	+
Odontoid Hypoplasia	+++	+++	+
Pectus Carinatum	+++	+++	-
Platyspondyly: vertebral bodies	+++	+++	-
Recurrent otitis media	++	++	++
Recurrent respiratory infections	++	++	+++
Rigidity of Joints (shoulder, elbow)	+	+	++
Scoliosis	+++	+++	-
Short Neck	+++	+++	-
Short Stature	+++	+++	+++
Short Trunk	+++	+++	+
Sleep Apnea	+++	+++	+++
Sleep Disturbance	-	-	+++
Snoring	+++	++	++
Speech Delay	-	-	++
Umbilical Hernia	+	+	++
Visual Impairment	+++	+++	+
Waddling Gait	+++	+++	-

Table 1: Differential diagnosis between MPS IVA, MPS IVB and MPS II.

Disorder	Deficient Enzyme	Chromosome	Storage Material
MPS I (Hurler)	α -L-Iduronidase	4p16.3	HS,DS
MPS II (Hunter)	Iduronate-2-sulfatase (IDS)	Xq28	HS,DS
MPS IIIA (Sanfillipo A)	Heparan-N-sulfatase	17q25.3	HS
MPS IIIB (Sanfillipo B)	α -N-Acetylglucosaminidase	17q21	HS
MPS IIIC (Sanfillipo C)	α -Glucosaminidase acetyltransferase	-	HS
MPS IIID (Sanfillipo D)	N-acetylglucoseamine 6-sulfatase	12q14	HS
MPS IVA (Morquio A)	Galactose 6-sulfatase	16q24.3	KS,C6S
MPS IVB (Morquio B)	β -Galactosidase	3p21.33	KS
MPS VI (Maroeaux-Lamy)	N-Acetylgalactoseamine-4-sulfatase	5q13.3	DS,C4S
MPS VII (Sly)	β -D-Glucuronidase	7q21-q22	HS,DS,C4,6S

Table 2: Except MPS IIIC, all genes related to MPS were identified (adapted from Educational CD for Hunter Syndrome) [70].

1.5 Glycosaminoglycans (GAGs)

Glycosaminoglycans (GAGs) are negatively charged linear polysaccharides composed of a linear chain of disaccharides with variable sulfation levels [71–75]. These disaccharides are made up one amino sugar (N-acetylglucosamine or N-acetylgalactosamine) and either a uronic acid (glucuronic acid or iduronic acid) or galactose sugar unit [76]. GAGs are one of the main mechanisms of the extracellular matrix (ECM) and play multiple roles in different tissues and organs. Polymeric GAGs are covalently bound through a linkage region to core proteins to produce proteoglycans (PGs) or remain as free polysaccharides [72,77–79]. PGs are associated with various physiological functions such as hydration and swelling pressure to the tissue to paying important roles in control of growth and differentiation [38]. Particular sulfation patterns in the GAGs allow interactions, of normally an ionic nature, with growth factors [76]. GAGs are accumulated in both MPS and mucopolipidosis (ML) [80].

In MPSs, GAG degradation pathways are disrupted due to lysosomal enzyme deficiency responsible for the degradation of GAGs: chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), keratan sulfate (KS), and hyaluronan. The degradation of GAGs is important to understand to establish new methods for screening and monitoring therapies. The degradation of GAGs includes four exoglycosidases, five sulfates, one nonhydrolytic transferase, and one endoglycosidase [76,81].

Enzyme deficiency causes undegraded GAGs to accumulate in multiple tissues leading to organ dysfunction representing a large variety of clinical signs and symptoms such as skeletal dysplasia, short stature, mental retardation, corneal clouding, hearing loss, heart valve disease, hepatosplenomegaly, and umbilical and inguinal hernias [82]. The accumulation of undergraded GAGs along the upper airway induces enlarged tongue, adenoidal, tonsillar, and vocal cord hypertrophy, and large mandible. GAG accumulation in the lower airway and cervicothoracic part results in an imbalance of growth between spine, rib, and manubrium vs. trachea and vessels in thoracic inlet causing consequent bulging and twisted narrowing trachea [83]. Although clinical manifestations do not usually appear at birth, the accumulation of GAGs can be detected in the human fetus for MPS I, II, III, IVA and in the placenta of MPS II, VI [84–86]. Accumulated GAGs alter connective tissue and cartilage, which changes the acquirement of bone mass and disturbs the microarchitecture of cartilage [87].

Establishment of GAG measurement facilitates diagnosis, prediction of clinical severity, prognosis, therapy monitoring (biomarker) and disease screening [88]. The evaluation of total levels of GAG in urine, cerebrospinal fluid or even in a cell culture can provide a suitable biomarker for MPS, which is important for diagnosis, monitoring the disease progress and therapy [89].

1.6 Aims of This Study

The broad goal of this research was to evaluate the procedure and methods for diagnosis and prognosis of patients with MPS. I have explored the following three aims.

1. Aim 1; To assess epidemiology of mucopolysaccharidoses.

Hypothesis: Incidence of MPS patients will vary between ethnic regions and countries.

2. Aim 2; To review the current diagnosis and prognosis for Morquio A syndrome

Hypothesis: Diagnosis and prognosis of Morquio A syndrome will require to use multiple evaluation methods.

3. Aim 3; To evaluate potential biomarkers in MPS.

Hypothesis: Pro-inflammatory factors and glycosaminoglycans will be biomarkers to discriminate types of MPS and clinical severity.

Chapter 2

EPIDEMIOLOGY OF MUCOPOLYSACCHARIDOSIS

It is required to estimate the incidence of MPS in each region and country to avoid the misdiagnosis or undiagnosis. The first study on the frequency of MPS was performed by Lowry and Renwick in British Columbia in 1971 [6], which was later updated between 1990 and 2000 [7, 8]. After that, the key reports for the incidence of MPS were published in Europe and other countries [9-19].

The incidence of MPS has been extensively reported in European countries although there have been several reports from Asian countries including Taiwan, China, South Korea, and India. Newborn screening for MPS is becoming more available to find early treatment options and consequently to improve activity of daily living. In this article, we presented the first data on the birth prevalence of MPS in Japan and Switzerland and compared these new data with results published for other countries to show the range of birth prevalence worldwide.

2.1 Birth prevalence vs. Incidence of MPS

Birth prevalence is calculated by the number of MPS cases during a period divided by the total number of live births during the same period and expressed as cases per 100,000 live births [18, 19].

Incidence is the number of newly diagnosed cases of MPS divided by the number of people that are at risk for the disease [90].

2.2 Birth Prevalence of MPS in Japan:

We investigated the birth prevalence of MPS patients in Japan between 1982 and 2009. Diagnosis of MPS was made in a high-risk population (suspected to be MPS) as described below.

2.2.1 Method:

Screening of Urinary GAG: The screening of urinary GAG occurred across the country for 18 years from 1982 to 1999 was performed at Gifu University. The urine specimen was sent to Gifu University when the patient was suspected as MPS clinically by the local physician. There was a blank period from 2000 to 2003 when data were not accumulated. In 2003, the urinary analysis was taken over by Special Reference Laboratories, INC (SRL), and interpreted by Dr. Orii (Department of Pediatrics at Gifu University, School of Medicine). The uronic acid quantitative test was carried out by acid carbazole method as described [20].

2.2.2 Results:

Number and type of MPS patients (1982-1999): In total, 331 cases were diagnosed for all MPS types. Different MPS during this period were; MPS I (14.8%, n = 49), MPS II (53%, n = 176), MPS III A (8.5%, n = 28), MPS III B (8.2%, n = 27), MPS III C (3.3%, n = 11), MPS IVA (9%, n = 30), MPS VI (1.2%, n = 4) and MPS VII (1.8%,

n = 6). MPS II accounted for over 50% of all types of MPS, followed by MPS I. Other types of MPS were a relatively lower percentage as described above.

Number and type of MPS patients (2003-2009): The specimen that has been requested from all over Japan by the methods described above. 136 patients were diagnosed in seven years between 2003 to 2009. Occurrence of MPS I (16.2%, n = 22), MPS II (58.1%, n = 79), MPS III A (3.0%, n = 4), MPS III B (8.1%, n = 11), MPS IVA (11.0%, n = 15), MPS IVB (0.7%, n = 1), MPS VI (2.9%, n = 4) was similar to that measured between 1982 and 1999.

The combined birth prevalence was calculated by the number of MPS cases diagnosed, divided by the total number of live births, which was 1.53 per 100,000 live births between 1982 and 2009. MPS I case was found 1 out of 430,000, MPS II (1 out of 119,000), MPS III (1 out of 385,000), MPS IIIA (1 out of 1,000,000), MPS IIIB (1 out of 834,000), MPS IIIC (1 out of 2.5 million), MPS IVA (1 out of 667,000), MPS IVB (1 out of 33 million), MPS VI (1 out of 3.3 million), and MPS VII (1 out of 5 million). The birth prevalence of MPS I, II, III, IV, VI, or VII was 0.23, 0.84, 0.26, 0.15, 0.03, or 0.02 per 100,000 live births, respectively. MPS II accounted for more than half of all MPS. MPS I, MPS III, and MPS IV were 15, 16, and 10%, respectively. MPS VI and VII accounted for only 1.7 and 1.3%, respectively, as shown in Fig. 6.

2.3 Birth Prevalence of MPS in Switzerland

Materials and subjects: The Swiss working group for Lysosomal Disorder (SALS)

established a national registry for patients with lysosomal disorders in 2009. This electronically based registry is technically supported by a non-university institution, and all the data are saved on an external and secure server. Informed consent of the patients or their parents, in accordance with requirements of the Local Ethics Committees of the referring centers, was obtained. The study was approved by the Local Ethics Committees. The retrospective study was carried out to identify all diagnosed MPS patients in Switzerland between 1975 and 2008. Patients enrolled were diagnosed either by measurement of reduced enzyme activity in leukocytes or fibroblasts and/or by molecular analysis. Abnormal urinary quantitative or qualitative GAGs were not satisfactory for a final diagnosis but were performed in all cases. Diagnosed patients are all followed in dedicated university centers (Bern, Lausanne, and Zurich) and were thus identified through these centers. Data collection was performed by a master student at the 3 centers. Data collection was based on the medical records.

The data were analyzed descriptively and the frequency of different subtypes of MPS determined. The age of patients at the onset of symptoms and diagnosis was used to calculate the pre-diagnostic interval. The registry was used for identification of the birth prevalence of the MPS patients.

Results: Switzerland has an average 85,000 births/year. In total, 51 patients with MPS were identified between 1975 and 2008 (34 years). 10 patients could not be enrolled in the registry as they were deceased. Out of 41 patients that were enrolled in the study, 2 died during the study (MPS III and MPS VI). A total of five patients had MPS I (female 1; male 4); 12 MPS II (male), 10 MPS III (female 5; male 5), 10 MPS IV (female 9; male 1), 3 MPS VI (female), and one MPS VII (male). The average age of onset of symptoms was 8 months for MPS I, 40 months for MPS II, 21 months for MPS III, 18 months for MPS IV, and 31 months for MPS VI respectively. The average age at diagnosis was 15 (MPS I), 56 (MPS II), 54 (MPS III), 38 (MPS IV), and 69 (MPS VI) months, respectively. The total live birth during this period was 2,621,036. The combined birth prevalence for diagnosed MPS was 1.56 per 100,000 live births. MPS II had the highest birth prevalence of 0.46 per 100,000 live births. Birth prevalence of MPS I, III, IV, VI, and VII was 0.19, 0.38, 0.38, 0.11, and 0.038, respectively. MPS II comprised 29% of all MPS, and MPS III and MPS IV each accounted for 24%. MPS I accounted for 12%. MPS VII showed the lowest incidence in the Swiss MPS population as shown in Fig. 6.

2.4 Other countries

Method: We have extensively searched the literature for incident and prevalence of MPS in different parts of the world and summarized in this section. The criteria for the diagnosis of MPS patients was made by the clinical manifestation and clinical and

molecular tests. The general diagnosis was made by elevated urinary GAGs and/or enzyme assay in serum, leukocytes, and/or fibroblasts.

2.4.1 Birth Prevalence of MPS in Saudi Arabia

In Saudi Arabia, Saudi Aramco Medical Services Organization (SAMSO) provided comprehensive free health care for its employees and their dependents. The data of 26 years from 1983 to 2008 was collected from SAMSO. The total number of live births in the study period was 165,130, and 248 cases were found with various metabolic diseases. The number of MPS diagnosed was 28 cases [21]. The birth prevalence of both MPS I and MPS IV was 3.62 per 100,000 live births, and each accounted 21% of all MPS. MPS VI had the highest estimated birth prevalence of 7.85 per 100,000, live births comprising 46% of all MPS cases diagnosed. The birth prevalence of MPS III was comparatively low (1.8) and accounted for 11% as shown in Fig. 6. No case for MPS II and VII was associated during this period.

2.4.2 Birth Prevalence of MPS in Taiwan

The diagnosis of MPS in Taiwan was made during a period of 21 years between 1984 and 2004. The data of live births were obtained from Department of Statistics, Ministry of the Interior, Taiwan. A total of 6,377,299 live births and 3,323,160 male live births were recorded during the period of study. 130 MPS cases were diagnosed with a combined birth prevalence of 2.04 per 100,000 live births [12]. The diagnosis of all patients was confirmed by two-dimensional electrophoresis of

urinary GAGs and/or enzyme assay in serum, leukocytes, and/or fibroblasts. The MPS II had the highest calculated birth prevalence of 1.07 per 100,000 (2.05 per 100,000 male live births), comprising 52% of all MPS cases diagnosed. Overall, the birth prevalence of all MPS was low and reported as 0.11, 0.39, 0.33, and 0.14 per 100,000 live births for MPS I, MPS III, MPS IV, and MPS VI, respectively. The percentage of MPS I, III, IV, and VI were 6, 20, 16, and 7%, respectively as shown in Fig. 6. In Taiwan, no case of MPS VII was reported.

2.4.3 Birth Prevalence of MPS in South Korea

In South Korea, 147 MPS patients were identified between 1994 and 2013 [22]. The combined birth prevalence of all MPS was 1.35. The birth prevalence of MPS II was 0.74 per 100,000 live births. The birth prevalence of MPS I, III, IV, and VI was 0.21, 0.25, 0.13, and 0.019 per 100,000 live births, respectively. The most common subtype of MPS was MPS II (54.6%), followed by MPS III (18.4%). MPS I and IV account for 15.3 and 9.5%, respectively. MPS VI was very low (1.4%), and no MPS VII has been identified until now [22].

2.4.4 Incidence of MPS in China

In China, the LSD study was carried out between 2006 and 2012. A total of 376 cases out of 1331 suspected patients were diagnosed for 17 different LSDs [23]. Patients in this study were from 21 provinces and municipalities in China, with 85.8% (n = 322) from East China. A small proportion of patients were from Northern China (n = 2), Northeast China (n = 3), Southwest China (n = 8), Northwest China (n = 9),

Southern China (n = 11) and Central China (n = 21). Among the LSDs, the most common group was MPS, representing 50.5%. In the MPS group, MPS II represented nearly half of all MPS cases diagnosed (47.4%) and a quarter of all diagnosed LSD cases, followed by MPS IVA (26.8%), I (16.3%), VI (4.2%), IIIA/B (3.7%), and VII (1.1%) [23]. Only one patient was diagnosed with MPS IVB. In the MPS group, three types (I, II, and IVA) constituted more than 90% of all MPS patients.

2.4.5 Incidence of MPS in India

Various LSDs was carried out by Sheth et al. in Indian population [24]. 1,110 children referred from various Indian states and a couple of neighboring countries (Sri Lanka and Afghanistan) from January 2002 to December 2012. It comprises of 938 (84.50 %) children from Western part of India, 121 (10.9 %) from Southern, 30 (2.7 %) from Northern, 1 (0.09 %) each from Eastern and central parts, 18 (1.62 %) from Sri Lanka, and 1 (0.09 %) from Afghanistan.

The majority of these LSDs were glycolipid storage disorders that include 48%; however, MPS included 22% of all LSDs [24, 25]. A total of 85 MPS cases were identified. MPS IV was the most common comprising 26%, followed by MPS I with 25%. MPS II, III, and VI accounted for 11, 12, and 21% respectively. MPS VII was the lowest among all MPS and accounted for 6%.

2.4.6 Birth Prevalence of MPS in Tunisia

In Tunisia, MPS patients were diagnosed based on urinary GAG and/or appropriate enzyme assay within the period of 1988 to 2005 which were considered for calculation of birth prevalence. Of the 132 suspected MPS, only 96 cases were confirmed as MPS [26]. The crude incidence rate was calculated including only 76 patients. MPS I; 22 (1/59,000), MPS II; 5 (1/346,000), MPS III; 24 (1/143,000), MPS IV; 15 (1/223,000), and MPS VI; 10 (1/333,000). Based on crude incidence rate the combined birth prevalence of all MPS (1/44,000 live births) was 2.27. The birth prevalence of MPS III was the highest among all MPS (0.7 per 100,000 live births), comprising 32% of all MPS cases diagnosed. The birth prevalence of MPS I, II, IV, and VI was 0.63, 0.29 (male live births), 0.45, and 0.30 per 100,000 live births respectively, which accounted for 25, 8, 21, and 13% of all MPS, respectively as shown in Fig. 6. No case for MPS VII was reported.

2.4.7 Birth Prevalence of MPS in Australia

In Australia, 188 MPS patients were diagnosed over a period of 17 years (1980-1996). The combined birth prevalence of all MPS was 4.46 per 100,000 live births [14]. The birth prevalence of MPS II, MPS IVA, and MPS VI was 0.74, 0.59, and 0.43 per 100,000 live births, respectively, which accounted for 17, 13, and 10%, respectively as shown in Fig. 6. The birth prevalence of MPS III was the highest, 1.51 per 100,000 live births, comprising 35% of all MPS. The birth prevalence of MPS I (1.14) was slightly lower than MPS III and accounted for 26% of all MPS. The birth prevalence of MPS VII was 0.047 per 100,000 live births and accounted for 1.1%. An

epidemiological study of MPS was carried out by Nelson et al. in Western Australia during 1969 and 1996. Only 22 MPS cases were diagnosed with the combined birth prevalence of 3.43 per 100,000 live births [16]. The birth prevalence of MPS III was very high 1.71 per 100,000 live births that accounted for 50% of all MPS. The birth prevalence of MPS I was 0.94 and accounted 27%. The birth prevalence of MPS II and MPS VI was 0.31 (0.61 male live births) and 0.31, respectively. The birth prevalence of MPS IVA (0.16) was comparatively lower than all other MPS. MPS II comprised 9%; however, MPS IVA and MPS VI comprised 4.5 and 9%, respectively, as shown in Fig. 6. In Australia, no case for MPS VII was reported.

2.4.8 Birth Prevalence of MPS in British Columbia, Canada

Lowry and Renwick [6] studied on the frequency of MPS in British Columbia, Canada in 1971. Lowry et al. and Applegarth et al. [7, 8] updated the study between 1990 and 2000. A total of 20 MPS cases were diagnosed over a period of 28 years between 1969 and 1996 [6-8]. The combined birth prevalence of all MPS was 1.94 per 100,000 live births. The birth prevalence of MPS I was the highest (0.58) among all MPS comprising 30%. The birth prevalence of each MPS II, III, IV, VI, and VII was 0.10 (0.19 per 100,000 male live births), 0.29, 0.39, 0.29, and 0.29 per 100,000 live births, respectively. MPS II, III, IV, VI, and VII accounted for 5, 15, 20, 15, and 15%, respectively as shown in Fig. 6.

2.4.9 Birth Prevalence of MPS in Brazil

The Brazilian Health System database reported 56,587,867 live births between 1994 and 2012 (19 years). A total of 600 cases of MPS I, II, IVA, and VI were diagnosed in this period (138 cases with MPS I, 220 with MPS II, 65 with MPS IVA, and 177 with MPS VI). This report [27] does not provide data for MPS III, MPS VII, and MPS IX. The combined birth prevalence was 1.04 per 100,000 live births. The highest birth prevalence was associated with MPS II which was 0.38 (0.75 per 100,000 male live births) that accounted for 37% of all MPS cases (not including MPS III, VII, and IX). The birth prevalence of MPS I, IVA, and VI was 0.24, 0.11, and 0.31 per 100,000 live births, respectively that accounted for 23, 11, and 30%, respectively, as shown in Fig. 6.

2.4.10 Birth Prevalence of MPS in the Czech Republic

In Czech Republic, LDS cases were diagnosed at the Institute of Inherited Metabolic Disorders in Prague between 1975 and 2008 (34 years). During this period, there were 4,261,897 live births. Out of 478 LDS patients, 119 patients were identified as MPS. The initial diagnosis was based on demonstration of accumulation of substrates, confirmed by the deficiency of relevant enzymes. The combined birth prevalence of all MPS was 3.72 per 100,000 live births [19]. The birth prevalence of MPS III was the highest, 0.91 per 100,000 live births that accounted for 20% of all MPS. The birth prevalence of MPS I and MPS IV was almost the same (0.72 and 0.73, respectively), which was slightly lower in the case of MPS II (0.43 or 0.83 male live births). The birth prevalence of unspecified MPS and multiple sulfatase deficiency

(MSD) was 0.6 and 0.26 per 100,000 live births. The birth prevalence of MPS VI and VII was very low (0.05 and 0.02 per 100,000 live births). The percentages of MPS I, II, and IV were 17, 18, and 13%, respectively. MPS VI and VII had a very low incidence as shown in Fig. 6.

2.4.11 Birth Prevalence of MPS in Denmark, Norway, and Sweden

In Denmark and Sweden, the study was performed over a period of 30 years between 1975 and 2004; however, the data for MPS cases in Norway was reported between 1979 and 2004 (26 years) [13].

2.4.11.1.1 Denmark

In Denmark, two laboratories, the Kennedy Institute in Glostrup and Department of Clinical Genetics at Rigshospitale, Copenhagen have performed the diagnosis for MPS until 2003, after that the later was used for diagnostic purposes. In Denmark, 33 MPS cases were diagnosed with the combined birth prevalence of 1.77 per 100,000 live births [13]. The birth prevalence of each MPS I, II, III, IV, and VI was 0.54, 0.27, 0.43, 0.48, and 0.05, respectively. MPS I, III, and IV accounted for 30, 24, and 27%, respectively. MPS II accounted for 15% and MPS VI for only 3%. No case for MPS VII was reported.

2.4.11.1.2 Norway

The diagnosis of MPS was carried out at the Department of Clinical Chemistry and Neurochemistry in Molndal, and more recently at the section for Genetics at Rikshospitalet, Oslo [13]. In Norway, 45 MPS cases were diagnosed with

the combined birth prevalence of 3.08 per 100,000 live births. The birth prevalence of MPS I was very high 1.85 accounting for 60%, followed by MPS IV (0.76) comprising 24%. The birth prevalence of MPS II (4%), III (9%), and VI (2%) was 0.13, 0.27, and 0.07 per 100,000 live births, respectively [13].

2.4.11.1.3 Sweden

Diagnosis of MPS was done by two laboratories, Center for Inborn Errors of Metabolism, Karolinska University Hospital, Huddinge, Stockholm, and Department of Clinical Chemistry and Neurochemistry in Molndal, Sahlgrenska University Hospital Gothenburg. Fifty-two cases with MPS were diagnosed with the combined birth prevalence of 1.75 per 100,000 live births. The birth prevalence of both MPS I and III was 0.67 comprising 38% each. The birth prevalence of MPS II was 0.27 that accounted 15%. The birth prevalence of both MPS IV and VI was low (0.07) comprising only 4% each. No case for MPS VII was found [13].

2.4.12 Birth Prevalence of MPS in Estonia

In Estonia, diagnosis of MPS was carried out between 1985 and 2006 (22 years) [11]. The data were collected from Children's Hospital in Tallinn and Department of Genetics of Tartu University Hospital. Selective screening of MPS was done by toluidine blue spot test [28] followed by quantitative analysis of GAGs in urine. When urinary GAG levels were elevated, enzyme analysis was performed to confirm the MPS. Only 15 MPS cases were identified with the combined birth prevalence of 4.05 per 100,000 live births [11]. A total of 370,298 live births were

listed in the study period. MPS II had the highest birth prevalence of 2.16 (4.2 per 100,000 male live births), followed by MPS IIIA with 1.62 per 100,000 live births. The birth prevalence of MPS VI was 0.27 per 100,000 live births. Among all MPS cases, MPS II contributed to 53% of all MPS. MPS III and MPS VI comprised 40 and 7%, respectively as shown in Fig. 6. In Estonia, MPS I, IV, and VII were not identified.

2.4.13 Birth Prevalence of MPS in Germany

A retrospective epidemiological survey study of MPS was carried out in Germany to estimate cumulative incidences of different types of MPS during a period of 16 years between 1980 and 1995 [9]. The data on the number of births during the study period was obtained from German Bureau of Statistics, and 13410924 live births were recorded. A total of 474 cases for MPS were identified, with the combined birth prevalence of 3.51 per 100,000 live births. The diagnosis of all cases was confirmed by enzyme assay in serum, leukocytes and/or fibroblasts [9]. The birth prevalence of approximately 0.69 per 100,000 live births was obtained for MPS I. The cumulative birth prevalence for MPS II was estimated 0.64 per 100,000 live births (1.3 per 100,000 male live births). The cumulative birth prevalence for MPS III was very high with 1.57 per 100,000 live births, compared to other MPSs. MPS IVA and MPS VI were found to be 0.38 and 0.23 per 100,000 live births, respectively. Overall, MPS III comprised 44%; however, MPS II and I were 18-20%. MPS IV and VI was about 11 and 7%, respectively as shown in Fig. 6. Relatively high number of patients with MPS

IIIB, IVA, and VI were of Turkish origin, and 617,013 Turkish children were born during the study period. The Turkish populations contributed to 10, 3, 6, 33, 22, and 52% for MPS I, II, IIIA, IIIB, IVA, and VI, respectively. No case for MPS VII was identified in Germany.

2.4.14 Birth Prevalence of MPS in the Netherlands

In the Netherlands, 331 MPS cases were diagnosed based on enzyme assay during a period of 27 years between 1970 and 1996 with the combined birth prevalence of 4.5 per 100,000 live births [18]. During this period, the number of live births for different MPS is shown in detail [18]. MPS I had the birth prevalence of 1.19 per 100,000 live births. The birth prevalence of all types of MPS III was 1.89 per 100,000 live births while the birth prevalence of MPS II was 0.67 per 100,000 live births (1.3 per 100,000 male live births). The birth prevalence of MPS IVA, IVB, VI, and VII was 0.22, 0.14, 0.15, and 0.24, respectively. The combined MPS III accounted for 47%, followed by MPS I and MPS II which occupied 25 and 15.5%, respectively. MPS IV accounted for 8%; however, both VI and VII comprised 2% as shown in Fig. 6.

2.4.15 Birth Prevalence of MPS in Northern Ireland

An epidemiological study of MPS was carried out in Northern Ireland over a period of 28 years between 1958 and 1985, and in total, 23 MPS cases were reported. The diagnosis was confirmed by appropriate enzyme assay [15]. There were 7 suspected Hurler syndrome cases that had died during the ascertainment period. All

children had typical clinical and radiological findings. They were also positive with urinary GAGs, and α -L-iduronidase enzyme activity showed intermediate values. There were also 4 MPS II cases that died during the ascertainment period before definitive enzymatic analysis [15]. During this period, 839,517 live births and 432,849 male live births were registered. The combined birth prevalence was 4.0 per 100,000 live births. MPS I had the highest birth prevalence of 1.66 per 100,000 live births followed by MPS IVA with 1.3 per 100,000 live births. The birth prevalence of MPS II and MPS III was 0.71 (1.39 male live births) and 0.36 per 100,000 live births, respectively. Among all MPS cases, MPS I was 41% followed by MPS IVA, II, and III, which were 32, 18, and 9%, respectively, as shown in Fig. 6. No case for MPS VI and VII was identified.

2.4.16 Birth Prevalence of MPS in Poland

In Poland, a retrospective epidemiological survey study of MPS was implemented to estimate birth prevalence. The live births registered in the Polish Bureau of statistics over a period of 4 decades were 21,686,890 cases from 1970 until 2010. A total of 392 cases were diagnosed with MPS with the combined birth prevalence of 1.80 per 100,000 live births [10]. Electrophoresis method was used for the initial diagnosis of MPS which was based on the demonstration of accumulated GAGs in the body fluids. Confirmation of various MPS was made by the deficiency of relevant enzymes. In Poland, MPS III had the highest birth prevalence of 0.86 per 100,000 live births, followed by MPS II, I, and IV with 0.46, 0.22, and 0.14 per

100,000 live births, respectively. The birth prevalence of unspecified MPS was 0.11 per 100,000 live births. The birth prevalence of MPS VI was very low (0.0132) as compared to other MPSs. Among all MPS cases, MPS III accounted for 48%. MPS I, II, IV, and VI accounted 12, 25, 8, and 1% respectively as shown in Fig. 6. No case for MPS VII was reported in Poland.

2.4.17 Birth Prevalence of MPS in Portugal

In Portugal, 62 MPS cases were identified over a period of 20 years from 1982 until 2001 [17]. The combined birth prevalence was 4.8 per 100,000 live births. The diagnosis was determined by urinary GAGs and confirmed by enzyme activity in the blood sample. The birth prevalence of MPS I was 1.33 per 100,000 live births and accounted 13%. The birth prevalence of MPS II, III, IVA, and VI was estimated as 1.09, 0.84, 0.6, and 0.42 per 100,000 live births, respectively. The birth prevalence of MSD was 0.48 per 100,000 live births. MPS II comprised 34% of all MPS followed by MPS III (23%), VI (16%), and IVA (10%) as shown in Fig. 6.

2.4.18 Birth Prevalence of MPS I in the United States

In the United States, two States, Illinois and Missouri, have implemented newborn screening for MPS I which is based on measurement of IDUA enzyme activity level in dried blood spot (DBS) specimens.

In addition, Kentucky and Pennsylvania have also have developed the NBS (<http://www.babysfirsttest.org>). University of Washington group also reported an assessment of MS/MS LSD-multiplex screening methods on anonymous DBS, with

follow-up genetic testing on these same DBS samples [29, 30]. Published reports of population-based pilot newborn screening for MPS I with diagnostic confirmation have come from programs in Italy and Taiwan [31, 32].

2.4.18.1.1 State of Illinois

According to Illinois NBS program contacts, from November 2014 through December 18, 2014, 17,300 newborns were screened [30]. Of the 17,300, 17 newborns were called out (0.1%) and repeated in triplicate before reporting. Referral results were as follows: no confirmed MPS I, 15 false positives, and 2 pending.

2.4.18.1.2 State of Missouri

In Missouri as of 2014, 174,636 DBS have been screened for MPS I disease [30]. There were 70 positive screens. Out of 70, they confirmed one severe MPS I patient, 58 false positives, 9 cases pending, and 2 newborns lost to follow-up. NBS results showed the birth prevalence of MPS I (of all types), 1.1 per 100,000 live births, in Illinois and Missouri [29].

2.4.18.1.3 University of Washington Study

Scott et al. [29] evaluated MS/MS multiplex screening procedures for three LSDs anonymous DBS from the Washington State newborn screening program. For MPS I disease, a cutoff of IDUA activity $\leq 1.15 \mu\text{mol/h/l}$, corresponding to $\leq 32\%$ of the mean, was used. Of the 106,526 samples, 9 screened positive for low IDUA activity was found. Based on these findings, overall birth prevalence of infants who “may eventually develop clinical symptoms of MPS I disease” was 1/35,700 (95% CI:

1/43,000-1/11,100). Because of the lack of information on phenotype, studies of anonymous dried-blood spots are not substitutes for true clinical epidemiology.

Puckett et al. [33] described the preliminary incidence and birth prevalence of MPS in the United States using the database from the National MPS Society between 1995 and 2005. Population information was obtained from the National U.S. Census Bureau. The combined birth prevalence was found to be 1.2 per 100,000 live births. Individual birth prevalence of MPS I, II, III, IV, VI, or VII, was 0.34, 0.29, 0.38, 0.09, 0.05, or 0.05 per 100,000 live births, respectively. MPS III was the highest incidence, followed by MPS I, II, and IV accounting for 31.7, 28.3, 24.2, and 7.5% respectively. MPS VI and VII were 4.2% each as shown in the Fig. 6.

The overall birth prevalence and period of study has been summarized in Table 3. A demographic distribution of the combined birth prevalence of different countries around the world has been shown in Fig. 7A.

2.5 Discussion

In this study, we have explored the birth prevalence of MPS in two countries, Japan and Switzerland and compared our results with the known birth prevalence in other countries.

We have demonstrated that 1) the birth prevalence rate of all MPS is similar in Japan (1.53) and Switzerland (1.56 per 100,000 live births), 2) the incidence of MPS II in Japan is as high as that in other East Asian countries, 3) the incidence and birth

prevalence of MPS varies in each country and region, and 4) the high incidence and /or birth prevalence of some MPS is reflected by founder mutations.

The birth prevalence of MPS in Japan and Switzerland was lower, compared to most European countries including the Czech Republic (3.72), Estonia (4.05), Germany (3.51), Northern Ireland (4.0), Norway (3.08), Portugal (4.8), and the Netherlands (4.5) [9, 11, 13, 15, 17-19]. However, the birth prevalence of MPS in Japan was comparable to central European countries such as Denmark (1.77), Poland (1.80), and Sweden (1.75) [10, 13]. Some other Asian or African countries including Taiwan (2.04) and Tunisia (2.27) have a similar birth prevalence [12, 26]. The birth prevalence of MPS in British Columbia is also similar (1.94) to that in these countries [7]. The limitation in countries having a small number of MPS including British Columbia, Denmark, Estonia, Northern Ireland, Norway Sweden, and Switzerland is due to a small number of population. Therefore the data may not be very significant. In MPS, there are unique common mutations in different MPS genes. Some common mutations in a particular MPS directly correspond to the high incidence of that particular MPS in a certain region or ethnic background; however, these mutations are not equally distributed among different regions. The birth prevalence of MPS often correlates with specific mutations that can predict the phenotype.

The birth prevalence of MPS I in Switzerland is low (0.19) as compared to that in Japan (0.23). This finding is incompatible with the fact that the incidence of MPS I is higher in European countries such as Norway (account for 60%), Denmark (30%), Sweden (38%) [13], and Northern Ireland (41%) due to two common mutations,

p.W402X and p.Q70X, in the *IDUA* gene [15], and moderately high in Germany (19.5%) [9] and the Netherlands (25%) [18]. The frequency of p.W402X and p.Q70X mutations varies in different countries. The p.W402X mutation was the most common, with 38.8 % in Spain [42], and p.Q70X was the most common, with 39% and 62 %, respectively, in Poland and Scandinavia [42, 44]. Additionally, p.W402X and p.Q70X accounted for 39% and 30%, respectively, in the United States, showing the highest frequency [43].

Norway has the highest birth prevalence of MPS I with 1.85 per 100,000 live births. Other European countries including Northern Ireland (1.66), Portugal (1.33), and the Netherlands (1.19) have comparable birth prevalence. In Australia, the birth prevalence of MPS I is also high (1.14) comparable to European countries. In addition, British Columbia (0.58) and Tunisia (0.63) have a moderately high birth prevalence of MPS I comparable to other European countries such as the Czech Republic (0.72), Denmark (0.54), Germany (0.69), and Sweden (0.67). Moore *et al.* have reported an exclusive MPS I study based on the severity of disease and found 1.07/100,000 live births in England and Wales that are consistent with other European countries [131]. The newborn screening (NBS) for MPS I, in progress in some US states suggests an overall birth prevalence of 1.1 per 100,000 live births (data from Missouri and Illinois[30]). However, recent data [33] described the birth prevalence of MPS in the United States as 1.2 per 100,000 live births. MPS I had the birth prevalence of 0.34/100,000 live births similar to Brazil and Asian countries as described by Federhen *et al.* [27].

In contrast to the incidence of MPS I, it is of great interest that in East Asian countries such as China [23], Japan (current study), South Korea [22], and Taiwan [12], the incidence of MPS II accounts for about 50% of all MPS. The birth prevalence of MPS II in most European countries is between 0.4 and 0.99 per 100,000 live births but notably very high in Estonia (2.16) and Portugal (1.09). The birth prevalence of MPS II in Japan (0.84) is similar to that in Taiwan (1.07) and other East Asian Countries. The birth prevalence of MPS II in Brazil is lower (0.38), similar to Czech Republic, Denmark, Norway, Sweden, Switzerland, and Tunisia. In general, East Asian countries including China, Japan, and Taiwan have a high incidence of MPS II that could be due to common mutation R468 in *IDS* gene [23, 48-50]. In contrast, in South Korea, IDS-IDS2 recombination mutations were most frequently found in Korean patients with a severe phenotype [22]. Another common mutation included p.G374G splicing mutation causing an attenuated phenotype [22].

The incidence of MPS III is also very high in European countries including Estonia [11], Germany [9], Poland [10], Sweden [13], and the Netherlands [18] and also Australia [16]. The incidence of MPS III (IIIA; 20-30% and IIIB; 10-14%) is higher in Germany, the Netherlands, and Western Australia than other MPS subtypes [9, 16, 18].

The birth prevalence of MPS III in Switzerland is 0.38, but the birth prevalence in Japan is even lower with 0.26. Most European countries have a high birth prevalence of MPS III (all types) per 100,000 live births that include the Czech Republic (0.91), Estonia (1.62), Germany (1.57), Poland (0.86), Portugal (0.84),

Sweden (0.67), and the Netherlands (1.89). Australia also has a very high birth prevalence (1.51) of MPS III comparable to European countries. MPS IIIA is the most common subtype in Northern Europe [9, 15, 18, 57, 58, 61]. However, MPS IIIB is the most common subtype in Greece [64]. In addition, MPS IIID is the more common in the Netherlands, compared to other countries [18]. The similar birth prevalence of MPS III, 0.7 per 100,000 births, was also found in Tunisia. Heron et al. [132] carried out a study in France for MPS III patients and showed the incidence between 1990 and 2006 (period of 17 years), compared with Greece and United Kingdom. The birth prevalence of MPS III was 0.68 per 100,000 live births in France; however, in the UK, diagnosis of MPS III was evaluated in children born between 1990 and 2006, which was 1.16 comparable to other European countries listed above. In Greece, the birth prevalence was 0.97 per 100,000 live births for MPS III. The incidences of MPS III are higher in, Germany, the Netherlands, and Western Australia with the common p.R245H mutation in MPS IIIA gene. In contrast, the incidence of MPS IIIB in Japan is only 8%, less than of Western countries, although the p.R565P mutation is present as a common mutation.

The birth prevalence of MPS IV is also variable in different countries; the birth prevalence in Switzerland is 0.38, but low in Japan (0.15). The birth prevalence of MPS IV was high in the Czech Republic (0.73), Northern Ireland (1.3), Norway (0.76), and Portugal (0.6).

The birth prevalence of MPS VI in Switzerland was low (0.11), comprising only 7.3% of all MPS and comparable to most of the European countries. However,

the birth prevalence of MPS VI in Japan was very low (0.03). Brazil has a high relative incidence of MPS VI (18.48% of all MPS) [133]. Moreover, Brazil had a higher birth prevalence of MPS VI (0.31) than most European countries but similar to Estonia, Tunisia, and West Australia [27]. There is a common mutation, 1533del23, among Brazilian MPS VI patients found in 23.1% of alleles, which also occurs in Portuguese MPS VI patients [111-116] that could reflect the high incidence of Brazilian MPS VI.

Jurecka *et al.* have reported MPS VI cases between 1983 and 2011 in Kazakhstan and Russia and compared with central and eastern European countries [134]. A high incidence of the p.R152W mutation was observed both in the whole series (42%) as well as in Russian patients (43%). The incidence rate ranged from 0.0363 to 0.64 per 100,000 live births in Poland and Lithuania, respectively.

MPS VII is one of the rarest MPS, along with MPS III D and MPS IX (which is the rarest one), with a very low birth prevalence. The prevalence of MPS is also influenced by the detection method in each country; in some cases with MPS III and IV, DMB-based spectrophotometry can give rise to false negatives, and therefore, it is important to consider other methods such as enzyme activity in serum, leukocytes, and/or fibroblasts.

To obtain more accurate incidence and birth prevalence of MPS, it will be needed to accumulate more cases in each type of MPS or to conduct comprehensive population screening (e.g. newborn screening) for MPS.

In conclusion, the overall birth prevalence of MPS (from 1.04 to 4.8/100,000) varies depending on the country and region or ethnic background. Some common mutations in specific regions directly contribute to the birth prevalence (or incidence) rate of each specific MPS. This information is important as specific treatment is available (or in clinical development) for most MPS types, making early diagnosis important to properly manage each case and positively modify the natural history of these debilitating diseases.

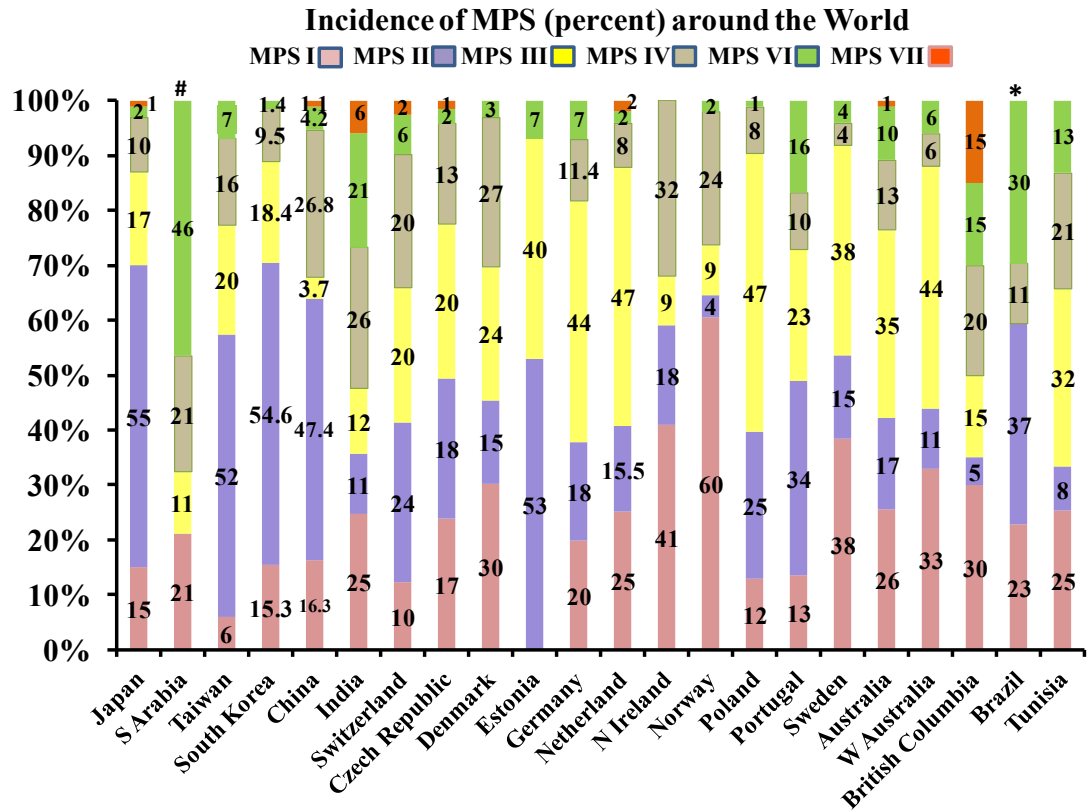


Figure 6: Incidence of MPS (%). *Brazilian report did not include data for MPS III and MPS VII. #Saudi Arabia has no MPS II data.

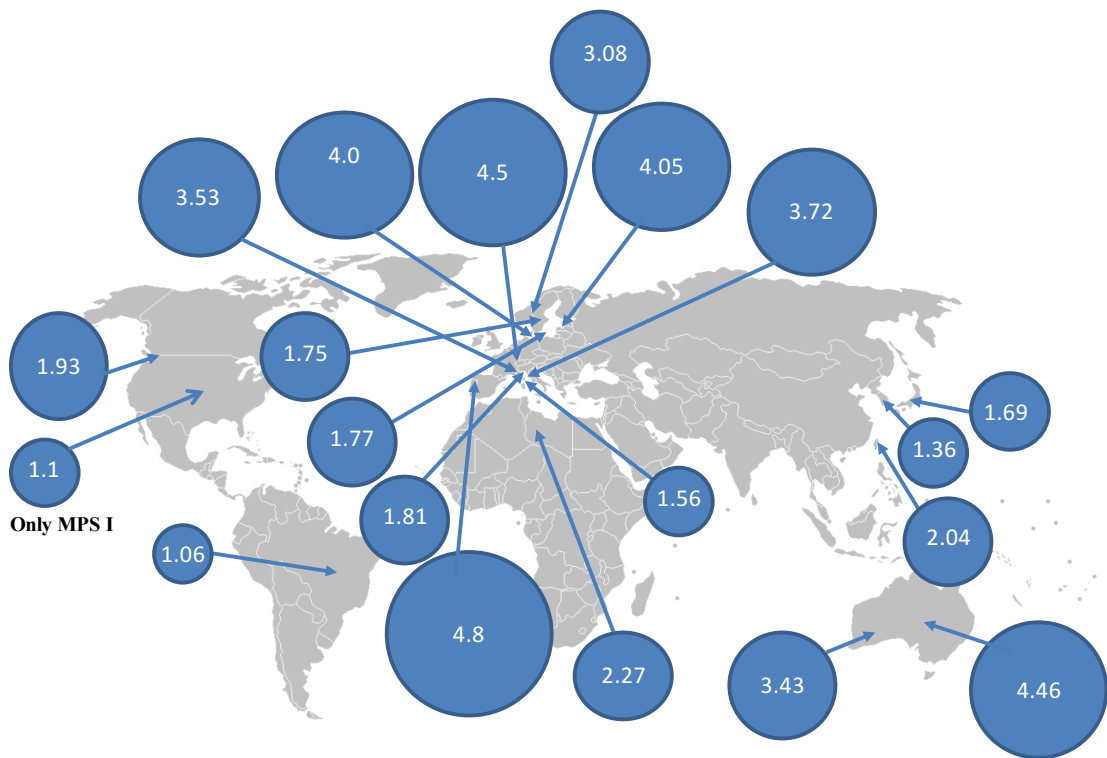


Figure 7: Demographic distribution of the combined birth prevalence of MPS. The combined birth prevalence of Europe is 3.14; the birth prevalence of individual countries is shown in Fig. 2B. The birth prevalence of Japan, South Korea, and Taiwan is 1.53, 1.35, and 2.04, respectively. The birth prevalence of Western Australia is 3.43; overall, Australian birth prevalence is shown as 4.46. The birth prevalence of Tunisia is 2.27. The birth prevalence of British Columbia, the United States, and Brazil is 1.94, 1.2, and 1.04, respectively.



Figure 8: Demographic distribution of the combined birth prevalence of MPS in Europe.

Table 3: MPS cases and prevalence in different countries by continent.

Country	Study period	Total MPS Cases	Combined Prevalence	Total Population at the end of Year	Reference
Africa					
Tunisia	1970-2005	96	2.27	10.03 M	Turkia <i>et al.</i> [2009]
Asia					
Japan	1982-2009	467	1.69	127.6 M	Present Study*
South Korea	1994-2013	147	1.36	10.8 M	Cho <i>et al.</i> [2014]
Saudi Arabia	1983-2008	28	8	26.37 M	Moammar <i>et al.</i> [2010]
Taiwan	1984-2004	130	2.04	22.75 M	Lin <i>et al.</i> [2009]
Australia					
Australia	1980-1996	188	4.46	18.31M	Meikle <i>et al.</i> [1999]
W Australia	1969-1996	18	3.43	1.9 M	Nelson <i>et al.</i> [2003]
Europe					
Czech Republic	1975- 2008	119	3.72	10.38 M	Poupetova <i>et al.</i> [2010]
Denmark	1975-2004	33	1.77	5.405 M	Malm <i>et al.</i> [2008]
Estonia	1985-2006	15	4.05	1.347 M	Krabbi <i>et al.</i> [2012]
Germany	1980-1995	474	3.53	81.68 M	Baehner <i>et al.</i> [2005]
Netherland	1970-1996	331	4.5	15.53 M	Poorthuis <i>et al.</i> [1999]
Northern Ireland	1958-1985	34	4	1.6 M	Nelson <i>et al.</i> [1997]
Norway	1979-2004	45	3.08	4.592 M	Malm <i>et al.</i> [2008]
Poland	1970-2010	392	1.81	38.18 M	Zurecka <i>et al.</i> [2015]
Portugal	1982-2001	353	4.8	10.36 M	Pinto <i>et al.</i> [2004]
Sweden	1975-2004	52	1.75	8.994 M	Malm <i>et al.</i> [2008]
Switzerland	1975-2008	41	1.56	7.648 M	Present study*
North America					
British Columbia	1969-1996	20	1.93	3.725 M	Applegarth <i>et al.</i> [2003]
South America					
Brazil	1994-2012	1069	1.06	200.4 M	Federhen <i>et al.</i> [abstract, 2016]

Chapter 3

DIAGNOSIS AND PROGNOSIS FOR MUCPOLYSACCHARIDOSIS IVA

In 1929, Dr. Luis Morquio, a pediatrician in Uruguay, first reported 4 Swedish siblings with unique skeletal dysplasia, later classified as Morquio A syndrome (MPS IVA). Those patients reported had disproportionate short-trunk dwarfism, hypermobile joints, prominent forehead, abnormal face with a large mandible, short neck, pectus carinatum, kyphoscoliosis, flaring of the rib cage, genu valgum, and pes planus [91]. At that same year, Dr. Brailsford, a radiologist in the UK, also reported a patient with similar skeletal manifestations, except that aortic valve disease and corneal clouding were not described in the original publication [92]. In 1962, Pedrini et al. reported urinary excretion of KS in three patients with Morquio syndrome, showing that this metabolic disorder differs from other mucopolysaccharidoses [93]. In 1965, McKusick et al. classified Morquio as MPS IVA [94]. Dr. Orii et al. reported patients with milder forms of MPS IVA [54,95].

These reports have demonstrated that patients with MPS IVA have a wide range of clinical heterogeneity and that the diagnosis of MPS IVA, especially with the attenuated form, can be difficult and delayed. Clinical phenotype and consequent prognosis are primarily determined by the extent of imbalance of growth between bones (flat bones - skull, hip, sternum, ribs; spine; short and long bones; sesamoid

bones), trachea, vessels, and organs, leading to serious morbidity and mortality with spinal cord compression and restrictive lung and obstructive airway [54].

3.1 Clinical Diagnosis

Diagnosis of MPS IVA begins with the development of unique clinical manifestations, which can occur from either clinical findings and/or radiographic findings [96]. Some symptoms that can be noticed at birth include prominent forehead, pectus carinatum, kyphosis (gibbus), and abnormal findings in the lumbar vertebral bodies in X-rays [12-15]. Gibbus, in particular, is often the first sign noticed in MPS IVA [97]. Diagnosis of MPS IVA patients is often not made until a few years of age since the majority of patients seem normal at birth, and normal levels of total urine GAG are observed in some cases [98].

GAG accumulation along the upper airway induces enlarged tongue, adenoidal, tonsillar, and vocal cord hypertrophy, and large mandible. GAG accumulation in the lower airway and cervicothoracic part results in an imbalance of growth between spine, rib, and manubrium vs. trachea and vessels in thoracic inlet causing consequent bulging and twisted narrowing trachea [83]. Respiratory involvement is seen with recurrent respiratory infections, upper and lower airway obstruction, tracheomalacia, restrictive lung disease, shortness of breath, loud snoring, look up to the sky position (head and neck maintained in extension), and sleep apnea [99]. Unlike other types of MPS, hypermobile joints (fingers, wrists, neck, and knees) are commonly observed;

however, decreased joint mobility can be observed in the large joints including shoulder, elbows, and hips [24].

There is a broad phenotypic spectrum in MPS IVA ranging from a rapid progressive early-onset form (severe) to a slowly progressive late-onset form (attenuated) [54]. In patients with the severe phenotype of MPS IVA, spinal cord compression, airway compromise, and later valvular heart disease are the leading causes of high morbidity and mortality, contributing to the shortened lifespan of individuals to the first few decades of life if untreated [83]. The severe form is characterized by hypermobile wrist joints, kyphosis, scoliosis, pectus carinatum, deformity of vertebral bodies, disproportionate dwarfism with short trunk, knock knee (genu valgum), and waddling gait [54]. Due to the progressive and often life-threatening nature of the disease [100], early and accurate diagnosis is critical for optimal patient management [96], which provides a better quality of life and prolonged lifespan [101]. Respiratory failure has been shown as the primary cause of death in patients (63%), followed by cardiac failure (11%), post-traumatic organ failure (11%), complications of surgery (11%), and myocardial infarction (4%) [101].

Patients with the attenuated form of the disease can survive for over 70 years if well-managed [102] and might have late-disease onset during late childhood or adolescence, and often first manifests hip problems (pain, stiffness) as the primary symptom [54] (Fig. 3 and 4). Clinical features are limited to minor skeletal

abnormalities, hip pain, and moderate short stature associated with the early-onset osteoporotic phenotype [103].

According to the International Morquio A Registry, the most common initial signs are short stature (49.9%), genu valgum (45.1%), kyphosis (44.4%), pectus carinatum (43.6%), and abnormal gait (37.8%). The most common current symptoms and the percentage of the present signs and symptoms are short stature (84.7%), genu valgum (78.7%), pectus carinatum (71.4%), kyphosis (70.4%), abnormal gait (64.4%), and laxity of wrist joints (63.2%) [55] (Fig. 9).

As the disease progresses regardless of the phenotype of the disease, more signs and symptoms begin to appear. With increasing age, joint laxity and skeletal deformities become more evident, and patients typically require support by orthopedic interventions [102]. The combination of bone and joint impairment leads to pain and arthritis causing the subsequent inability of walking, restriction of range of motion, and consequently decreased activity of daily living [54].

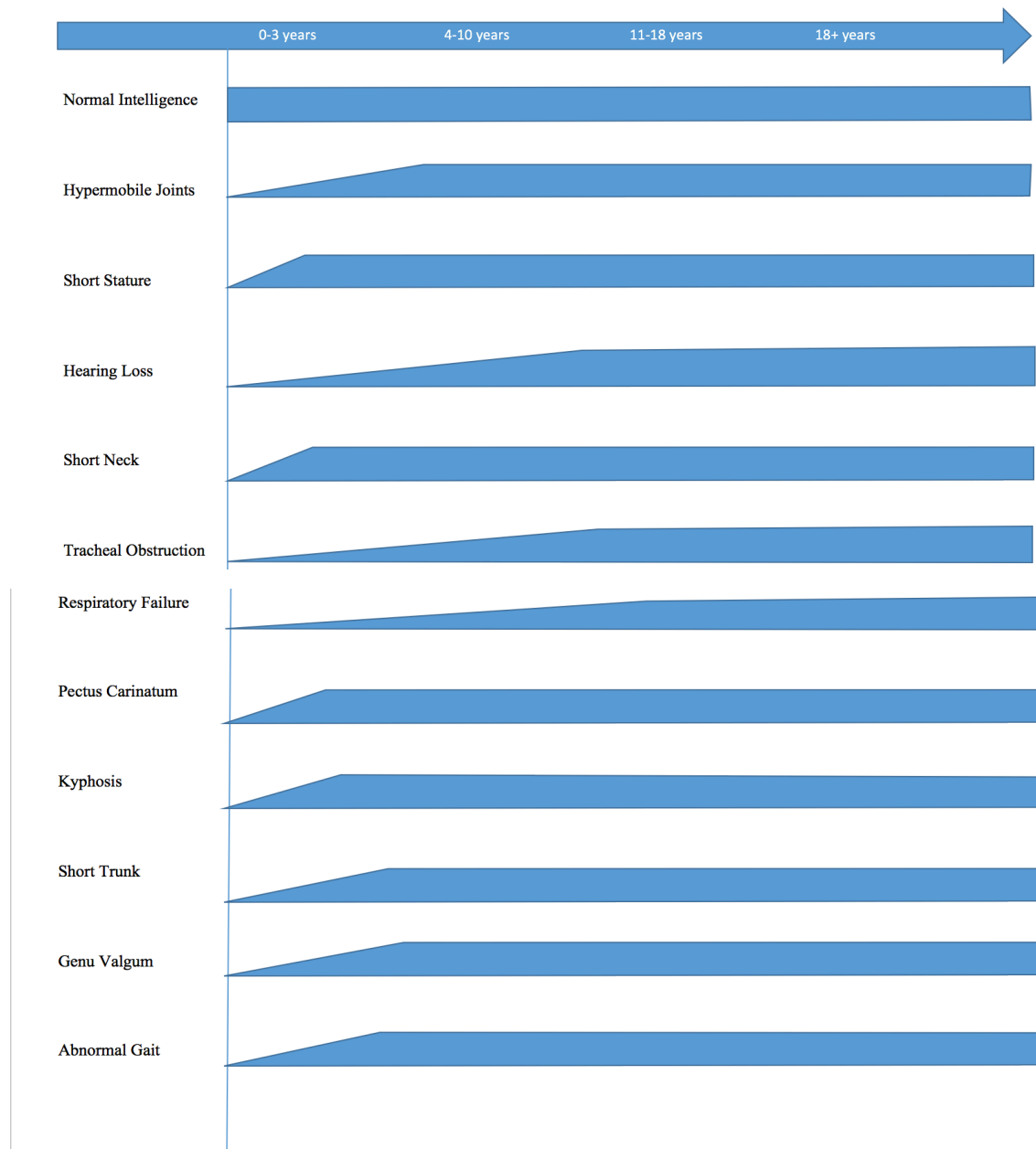


Figure 9: Time course of clinical symptoms in MPS IVA

3.2 Clinical Diagnosis

3.2.1 Urinary and Blood GAG

Urine-based testing for total GAG is considered only a screen and provides substantial false negatives; it is recommended that KS measurement, as well as enzymatic testing, are used to confirm the diagnosis of MPS IVA [55]. Measurement of urinary GAGs is performed both quantitatively and qualitatively [55]. While a gross elevation of urinary GAGs is normally specific for patients with other types of MPS, borderline or slightly elevated values are common in those with MPS IVA [104–107].

False negative results are found for almost 15% of all MPS patients [106] and even more for MPS IV patients [104–106]. The urinary excretion of GAGs is high in infants and young children, decreases with age, plateaus by the second decade of life, and remains constant through adulthood [105,106,108–110]. The qualitative analysis of total urinary GAGs is usually performed by spectrophotometric analysis using dimethylmethylene blue (DMMB) [111]. For qualitative urine-based testing methods, the GAGs are first isolated from the urine and then separated by thin layer chromatography or electrophoresis [112–115].

3.2.2 Keratan Sulfate (KS)

KS is mostly synthesized and accumulated in the cartilage and cornea in MPS IVA patients, and excessive accumulation of KS in lysosome leads to disruption of

chondrocytes and subsequent increase of KS levels in blood and urine [70]. We had two autopsied cases. The first autopsied case died of respiratory complication with tracheal obstruction after cervical fusion at 20 years old [35]. The second case died of respiratory failure with tracheal obstruction as well. In both cases, the chondrocytes in various bones had accumulation of storage materials [116]. We have analyzed over 20 cases of surgical remnants with various bones. All chondrocytes were vacuolated and were severely affected [83,87,116,117]. Primary synthesis of KS in chondrocytes and successive storage of KS in chondrocytes because of the deficiency of GALNS enzyme proves the cartilage as the main source of KS in blood circulation.

The detection of KS in biological specimens with tandem mass spectrometry (MS/MS) has been developed first by Oguma et al. [118] and applied to specimens in MPS IVA patients and distinguished between control subjects and MPS IVA patients without the false negatives [119–121]. Blood and urine KS levels are higher in MPS IVA patients, compared with age-matched controls [12, 37–39]. Urine KS levels remain higher or subnormal in MPS IVA patients than those in controls after 20 years of age; however, blood KS levels tend to be normalized by the age of 20 years [119–121].

Using sandwich ELISA [104,122] and LC/MS/MS [118,120] assay, the study was conducted for KS levels in blood and urine from MPS IVA patients and healthy controls to evaluate the comparability of results. Blood, urine, and other biological samples were purified by filtration and GAGs are digested with chondroitinase ABC

and keratanase II to yield disaccharides of C6S and KS, respectively. After digestion, the samples were injected into the LC-MS/MS [119,121,123–126].

The levels of blood and urine KS correlate with clinical severity during the early and progressive stage of the disease, and therefore, it is a good prognostic biomarker at this stage [98]. Blood KS directly displays growth, turnover disruption, and/or repair of cartilage where it is mainly synthesized [98]. The advantage of measurement of KS in dried blood spot (DBS) testing is its convenience for transport of samples and screening purposes [98]. In contrast, urine KS has a broader range of value and may not reflect cartilage condition directly. KS is synthesized mainly in cartilage. Depending upon the extent of destruction of cartilage in MPS (not only IVA but other types), KS level was increased in blood [98,104,127]. The levels of blood and urine KS also correlated with clinical severity during the early and progressive stage of the disease [104] and therefore, it is a good prognostic biomarker at this stage. Urine KS comes via kidney, and urine KS is filtered in kidney. Therefore, only selected smaller molecules are excreted in urine [98,128]. When MPS IVA patients are treated with ERT, it is likely that urine KS levels are reduced rapidly since the enzyme is delivered to kidney and digests the KS stored in the kidney [129]. Thus, urinary KS is useful to differentiate MPS IVA from other forms of MPS and to demonstrate pharmacodynamic effects of therapy while it does not provide a valuable predictor (biomarker) of skeletal or clinical improvement during these therapies for MPS IVA [130]. It is noteworthy that the origin and character of urinary GAGs are different from those of blood GAGs as seen is KS [131,132].

The ratio of di-sulfated KS to total KS in urine is much higher than that in blood [133]. There is a negative correlation between blood and urine for levels of mono- and di-sulfated KS in ERT-treated MPS IVA patients, suggesting reduction of urine KS level does not reflect blood KS level [133].

GALNS plays a role as galactose-6-sulfatase (G6S) because the enzyme hydrolyzes the sulfated galactose of KS and converts di-sulfated KS to mono-sulfated KS [130,133]. Therefore, deficiency of GALNS activity leads to the accumulation of more di-sulfated KS, and therefore, the ratio of di-sulfated KS to total KS of patients with MPS IVA increases, compared with normal controls especially at an early stage [130]. Levels of both mono-sulfated and di-sulfated KS in blood were measured by LC/MS/MS, and patients with MPS IVA had higher KS with both forms, compared with age-matched controls [134]. The elevation of di-sulfated KS in MPS IVA patients was more significant than that of mono-sulfated KS [130]. The proportion of di-sulfated KS vs. total KS in blood rose with age in control subjects while it was age-independent in patients with MPS IVA. The proportion of di-sulfated KS is better at distinguishing younger MPS IVA patients than older patients from age-matched controls. Levels of mono- and di-sulfated KS in the urine of MPS IVA patients were also higher when compared to those in age-matched controls for all studied ages. A significant difference in sulfation levels of KS between control subjects and patients with MPS IVA indicates that di-sulfated KS is another potential biomarker for MPS IVA [130].

Overall, determination of urine KS concentration provides a potential biomarker to screen for a high risk of MPS IVA patients and to measure pharmacodynamics effects [76]. This is important for assessing the clinical status at the initial progressive stage. Blood KS could be used for 1st tier newborn screening followed by the enzyme assay or vice versa [98] and is a potential biomarker to provide the clinical severity and therapeutic effect for the bone at the initial progressive stage [128].

3.2.3 Chondroitin-6-sulfate (C6S)

The GALNS enzyme plays an important role in converting C6S to CS, by hydrolyzing the sulfate group in C6S. Measurement of C6S has been developed by LC/MS/MS [135]. C6S levels were assayed in the blood and urine of patients with MPS IVA and were significantly elevated compared with age-matched controls, and declined with age in both MPS IVA patients and control subjects [136]. The difference of C6S in the patients and controls were more pronounced than the increased levels of KS in patients with MPS IVA [136].

It is important that the data of C6S and KS are reported together, as the information provided enhances discrimination of patients from controls compared to C6S or KS levels alone [135,136]. Thus, C6S could be another useful biomarker for MPS IVA [135]. Overall, blood and urine KS and its sulfation level should be measured for diagnosis, the prognosis of the phenotype, and assessment of pharmacokinetic efficacy. Blood KS and/or C6S could be a biomarker for therapeutic

effect only at presymptomatic to the early progressive stage but not beyond the progressive stage.

3.2.4 Enzyme assay

In 1976, the enzyme deficiency in MPS IVA (GALNS deficiency) was identified [137]. GALNS acts on 2 substrates: N-acetylgalactosamine-6-sulfate (GalNAc-6S) [138] and galactose-6-sulfate (Gal-6S) [139,140].

GalNAc-6S is a component of C6S, and Gal-6S is a component of KS [138–140]. These substrates are both used currently to demonstrate deficient GALNS activity [96]. The GalNAc-6S based assay uses radio-labeled natural substrate [141], and the Gal-6S based assay uses a fluorogenic artificial substrate [142]. GALNS activity is determined based on the amount of radioactivity released from this substrate with a lack of GALNS activity, resulting in the low generation of the signal [142]. The fluorogenic assay uses the 4-methylumbelliferyl- β -D-galactopyranoside-6-sulfate (4MU-Gal6S) substrate [142]. GALNS present in the sample first removes the 6-sulfate, and then exogenous β -galactosidase removes the galactoside, freeing the 4-methylumbelliferone adduct, which will fluorescent under high pH [142]. The addition of exogenous β -galactosidase to the reaction mixture is critical because conditions in which β -galactosidase is deficient would result in significant GALNS activity underestimation and possibly misdiagnoses [142].

To facilitate prenatal diagnosis, prenatal samples, such as dissected chorionic villi, cultured chorionic villus cells, and amniocytes can also be used [143,144].

Fibroblasts and leukocytes are recommended for diagnosis of the deficiency in GALNS activity [145]. Protocols for evaluating GALNS activity in DBS samples have recently been proposed as screening methods [146,147]. Leukocytes that are isolated from whole blood are better for more rapid analysis as cell culture is not required [148].

Fibroblast samples are recommended for enzyme activity analysis due to the impact of environmental and logistical factors during shipment can be minimized and corrected through culturing of the cells [96]. The measurement of GALNS activity in DBS is useful for screening, but it is not as robust as it is in fibroblasts or leukocytes due to the low number of cells present in the sample [96]. More data is needed to evaluate GALNS stability in DBS because DBS samples are more likely to be unprotected to environmental extremes during shipping in comparison to leukocytes [96]. The activity of a reference enzyme with similar stability in the same sample should be measured to confirm that the low GALNS activity is not the cause of sample degradation [96]. If the DBS sample is used, measuring a reference enzyme in the same sample to confirm integrity is recommended, but it may not be enough to rule out an effect of handling on GALNS activity due to the stability of GALNS as compared to other enzymes in a DBS which remains unknown [96].

The MS/MS-based method using a novel substrate has recently been developed [149]. Incorporation of this new assay into a multiplexed lysosomal storage disease panel for use in newborn screening programs is being considered [150].

3.3 Radiographic diagnosis

3.3.1 Skeletal Manifestations

When MPS IVA is suspected, radiographic imaging should be performed as a component of the diagnostic process [96]. A patient should obtain a skeletal survey to allow evaluation of the skull, complete spine (including flexion-extension lateral views), chest, hips, and limbs (particularly, hands, wrists, and knees) because of the wide variation and subtleties of radiographic findings in MPS IVA [96].

3.3.2 Skull

In 8 out of 14 individuals with MPS IVA, subtle abnormal brain MRI findings such as prominent perivascular space, enlarged lateral ventricles, and prominent frontal cerebrospinal fluid (CSF) were reported [151].

3.3.3 Spine

Children with MPS IVA have instability of the neck, odontoid hypoplasia, ligamentous laxity, incomplete ossification of the anterior and posterior rings of the atlas, and spinal cord compression [98,100,152,153] (Fig. 10). Spinal cord compression may occur in any spinal segment; however, cervical spinal compression is the most common site [54]. Spinal cord compression may be due to several factors such as cervical instability, ossified fibrocartilage associated with an abnormal odontoid process, ligamentous laxity, cartilaginous and ligamentous hypertrophy at the atlantoaxial joint, GAG deposition in the extradural space, disc protrusion, kyphoscoliosis, and acquired central canal stenosis [102,154–157].

If MPS IVA is suspected or diagnosed in a patient, frontal and lateral radiographs of the spine should be evaluated [157]. Spinal involvement in MPS IVA occurs at 2 distinct sites; cervical and thoracic [157]. Cervical spinal involvement, particularly instability and compression at the C1-C2 level, is a nearly universal finding and predisposes patients to myelopathy, paralysis, and sudden death [102]. Knowledge of the unique anatomy and pathology of the atlantoaxial (C1-C2) system facilitates radiological detection of upper cervical spine anomalies [157]. Spinal cord compression at the cervicothoracic or the thoracolumbar level due to kyphotic deformity can lead to paraplegia with gradual onset and all of its devastating consequences, although it is not common [158,159]. A lateral cervical spine x-ray of a 6-year-old female patient showed hypoplastic odontoid, platyspondyly, and anterior subluxation on C7 on T1 [54]. If cervical spine instability is suspected on plain film or with inconclusive radiographic findings, then flexion-extension cervical spine MRI can be used to evaluate cord compression [54]. Flexion-extension CT with sagittal reformation and soft-tissue filtration showed cone-shaped dens, a thickened cruciate ligament, and small thick cartilaginous posterior arch of C1. With flexion, there is a narrowing of the canal between the body of C2 and the cartilaginous posterior arch of C1.



Figure 10: Lateral radiographs of the cervical spine in flexion (a) and extension (B) show atlantoaxial instability in an 8-year-old female patient with MPS IVA. The anterior arch of C1 moves anteriorly in flexion (arrow) in relation to C2. There is hypoplasia of the dens (dashed arrow) and generalized platyspondyly.

3.3.4 Upper Extremities

Upper extremities show the irregular epiphyses and widened metaphyses even at an early stage [136]. Characteristic radiographic findings in hand and distal forearms of individuals with MPS IVA include a short ulna, ulnar deviation of the radial epiphysis, and delayed maturation of the carpal bones; the scaphoid, however, may not be radiographically present [55,160]. Metacarpals may be short and the proximal ends of the second through fifth metacarpals are typically rounded or pointed [55,160]. The upper extremities involvement is progressive and affects the wrist strength in some activities of daily living [54].

3.3.5 Lower Extremities

The lower extremity involvement is universal and progressive if untreated [161,162]. The most common lower extremity deformities are knee and ankle valgus. Genu valgum results from distal femoral and proximal tibial involvement and joint laxity [54]. Children with MPS IVA have unique waddling gait, a slower walking speed, reduced cadence, and reduced stride length [163]. The hips are either normal or slightly subluxated to start and may progressively dislocate over time [98]. The capital femoral epiphyses are smaller and progressively flattened, and many become fragmented over time [98]. The articular cartilage is abnormal and will degenerate promptly and build up early arthrosis, especially in the lower extremities [98].

3.3.6 Hips

Early in the disease course, the capital femoral epiphyses are small, and the acetabuli are shallow [54]. Affected individuals can become wheel-chair bound due to the following gradual and destructive changes in the femoral head and acetabuli resulting in hip dislocation, arthritis, and severe joint restriction [102] (Fig. 11). Several abnormalities are observed in the pelvis, including dysplastic femoral heads and oblique acetabular roof with coxa valgus deformity and flared iliac wings [136]. A hip x-ray of an 8-year-old female showed a bilateral irregular flattening of the capital femoral epiphyses and irregular dysplastic acetabuli with lateral joint subluxation [54].

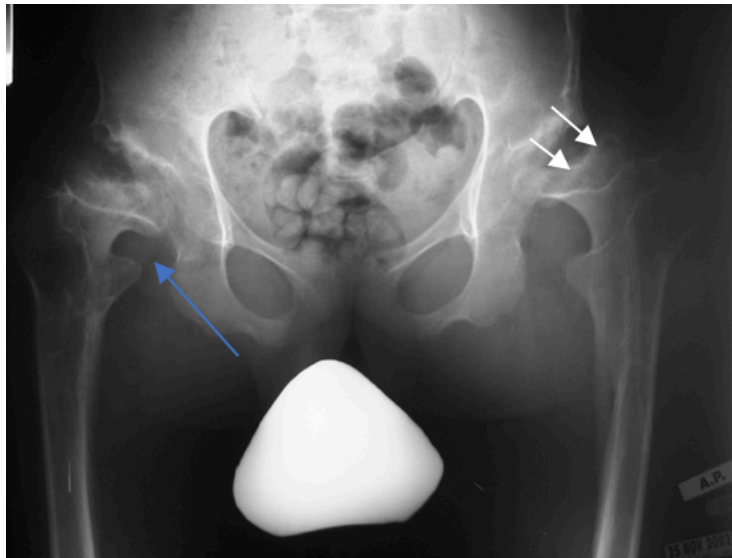


Figure 11: Erect frontal radiograph of the hips from a patient with MPS IVA. Shown here is progressive proximal and lateral migration (blue arrow) as well as the flattening and eventual disappearance of the proximal femoral epiphysis (white arrows).

3.3.7 Knee

The knee is the most commonly affected lower extremity joint in MPS IVA.

Laxity of the collateral ligaments aggravates the deformity [164]. The proximal tibia contributes to more knee deformity than the distal femur, and there may also be procurvatum of the distal femur with recurvatum of the proximal tibia [164].

Arthrograms or magnetic resonance imaging (MRI) of the knees in MPS IVA patients shows that the proximal lateral portion of the tibia is unossified and the fibula is short; these results offer clarity of the bony and cartilaginous nature of the deformity [102].

Knee valgus causes the ground force to be shifted laterally, which forces the knee to go into further valgus [164] (Fig. 12). Correction of knee valgus using guided growth, such as the 8-plate hemiepiphysiodesis, is proven to be effective as the child is growing [161]. Multiplanar and more severe deformities may be corrected with osteotomies around the knee [165].

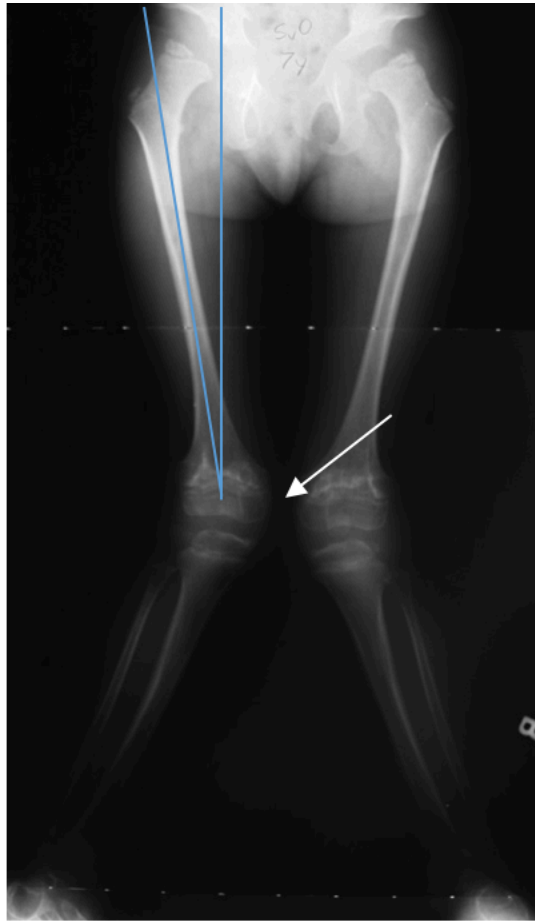


Figure 12: Erect frontal radiographs of the lower extremities of a patient with genu valgum.

3.3.8 Ankle

The ankles of MPS IVA patients are typically in valgus with wedging of the distal tibial epiphysis and shortening of the fibula [164]. The patients might have hindfoot valgus, some degree of equinus in the hindfoot, and adductus in the forefoot [164] (Fig. 13). Ankle valgus can be managed by orthotics but sometimes may require surgical correction such as guided growth or an osteotomy [161]. On lateral images, tarsal bones are irregular, and the talus appears to be plantarflexed [164]. Recurrence of the knee and/or ankle valgus is not uncommon in MPS IVA patients [166].

Imaging of x-rays, computed tomography (CT), and MRI is important for the diagnosis of MPS IVA because of the evaluation of cervical spine instability, stenosis, and cord compression [157]. Radiographs are the mainstay for assessing and monitoring lower extremity bone involvement; two dimensional CT provides detailed information on the bone structure and also allows for precise determination of the bony dimensions [164,167]. However, progressive fragmentation and collapse of the osseous structures can lead to false assumptions regarding the anatomy around joints [164]. To get a more accurate picture of the non-ossified structures and the articular surfaces, arthrography or MRI will be needed [164]. The cartilaginous part of the epiphysis is often not as deformed as could be predicted by the plain radiographs [164]. CT does not offer the ability to assess the health of the spinal cord itself [157]. The detection of canal stenosis, cord compression, and myelomalacia is critical to check for the disease progression [157]. MRI is the most useful method for evaluating the neural axis in MPS IVA and is also currently considered the imaging method of

choice for evaluating the spinal cord [157]. No other imaging method provides superior information about the soft tissues, including the cartilage, ligaments, dura, spinal cord, and nerve roots [157].



Figure 13: Feet of a patient with MPS IVA. Photograph of the feet (a) and lateral radiograph of the left foot (b) of this MPS IVA patient show skew foot posture in both feet (white arrows), pronated pes planus (orange arrow), and increased sandal gap between the first and second toes (black arrow).

3.3.9 Imbalance of Growth

MPS IVA is characterized by accumulation of GAGs in chondrocytes, the incomplete ossification, and the successive imbalance of growth, which cause unique clinical features of disproportionate short trunk dwarfism, hypermobile joints in hands and fingers, a prominent forehead, an abnormal face with a large mandible, short neck, cervical spinal cord compression, tracheal obstruction with crowd thoracic inlet, pectus carinatum, flaring of the rib cage, coxa valga, genu valgum, and pes planus [55,91,92,95,102,138,168,169].

The frontal bone, parietal bone, occipital bone, and the mandible keep growing, causing MPS IVA patients to have an enlarged head, a prominent forehead, and a large mandible. The cervical spine in the region C1-C7 halts growing earlier in patients with MPS IVA, causing the patients to have a short neck. Cervical spine shows platyspondyly and typical beaking of the anterior margin of the vertebral bodies, causing kyphosis as well as instability of the atlantoaxial joint and spinal cord compression (Fig. 14). Tracheal abnormalities which include stenosis, tortuosity ultimately resulting in obstruction is due to several factors, including the imbalance of growth in patients with MPS IVA. The thoracic spine from T1 to T12, as well as the lumbar region of the spine from L1 to L5, stop growing early causing patients with MPS IVA to have a short trunk. Pectus carinatum is marked deformity of the anterior chest wall because the ribs (costal cartilage) overgrow compared with other parts of the body, causing restrictive lung [170]. The wrist joints show marked hyperlaxity because the ulna stops growing earlier and the radius continues to grow, resulting in an

ulnar deviation of the wrist. The metacarpals and carpals grow slowly in patients affected by MPS IVA. The growth plates are wide with mild generalized brachydactyly and hypoplastic carpal centers [59] (Fig. 15). Hip deformities are caused by a combination of the bilateral flattening and fragmentation of the capital femoral epiphyses and acetabular dysplasia with lateral joint subluxation [171]. The pelvis shows wide flared iliac bones laterally [172]. Genu valgum is caused by the mechanical axis shift laterally, where pathologic stress is placed on the lateral femur and tibia which inhibits growth [173]. The lower limbs malalignment is evaluated with the measurements of the mechanical axis deviation (MAD) [174]. The patellofemoral joint may become shallow, incongruous, or unstable, causing activity-related knee pain in patients [173]. Patients will not only have knee pain and laxity, but they can develop a circumduction gait, in which each leg is swung outward to avoid knocking their knees together due to genu valgum [173].

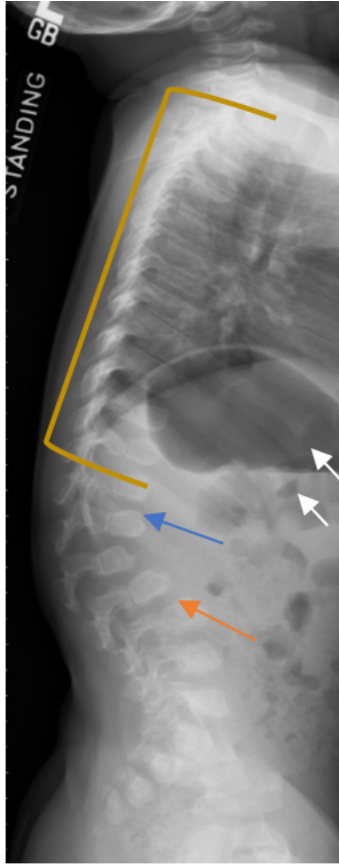


Figure 14: Lateral radiograph of the spine of a two-year-old MPS IVA patient with vertebral wedging (blue arrow). Platyspondyly (yellow line), anterior inferior beaking (orange arrow), rib flaring (white arrows) are seen in this X-ray radiograph of a patient with MPS IVA.



Figure 15: X-ray radiograph of an MPS IVA patient hand. Clearly seen are the tapering of the proximal portion for the metacarpals (red arrows), small irregular carpal bones (white arrow), and the distal portion of the radius being tilted toward the ulna (blue arrow).

3.4 Non-Skeletal Manifestations

3.4.1 Ophthalmology

The most common ophthalmologic findings are related with slowly progressive corneal clouding (Fig. 16). Generally, in patients with MPS, corneal opacification of varying severity is frequently seen, as well as retinopathy, optic nerve swelling and atrophy, ocular hypertension and glaucoma [175]. Other ophthalmologic findings that are less common are astigmatism, cataracts, punctate lens opacities, open-angle glaucoma, optic disc swelling, optic atrophy and retinopathy [166,176]. Ocular manifestations are common in MPS and may result in significant visual impairment [175]. Corneal opacification often causes reduced vision in the early childhood of MPS IVA patients, necessitating penetrating keratoplasty for which the outcome can vary [54]. In a study with 20 patients aged 1-65 years, with MPS IV, in which the subtype A or B was not specified, 10 eyes had no corneal clouding, 17 eyes had mild corneal clouding, 4 eyes had moderate corneal clouding, and 4 eyes had severe corneal clouding (corneal clouding was not graded in 5 eyes) [177]. The severity of corneal clouding was related to the increasing age of the patient and results in the reduction in visual acuity [177]. The corneal deposits can also interfere with examination of the other ocular structures, such as the trabecular meshwork, the retina, and the optic nerve [176].

While visual acuity is better in MPS IVA patients compared to other MPS patients, the corneal clouding, refractive errors, glaucoma, and cataracts can affect the

visual acuity as the patient ages [176]. Glaucoma or ocular hypertension seems to be unusual in MPS IVA [178]. Electron microscopy shows distended trabecular endothelial cells with a thickened basement membrane in MPS IVA [176]. The inclusions in the trabecular meshwork are primarily multi membranous, whereas other types of MPS inclusions are more fibrillogranular [179,180]. This most probably explains the reduced prevalence of ocular hypertension and glaucoma in individuals with MPS IVA [179,180].

Annual eye examinations should be performed in MPS IVA patients and should include the following: slit-lamp biomicroscopy of the cornea, measurement of intraocular pressure, assessment of refractive error, and examination of the posterior segment [176]. If the vision reduces, evaluation with low-vision aids should be considered [176].



Figure 16: Eyes of a patient with MPS IVA. Image of a 24-year-old patient with MPS IVA shows fine stromal corneal clouding (adapted from Educational CD for Morquio and permitted by Carol Ann Foundation).

3.4.2 Auditory system

Hearing impairment is common among patients with MPS IVA [54,97,100,176,181–183]. Conductive hearing loss caused by serous otitis media due to frequent upper respiratory tract infections may be present anytime from birth and onwards [9, 94]. Many patients with MPS IVA have one or more sets of ear pressure equalization tubes to treat conductive hearing loss due to recurrent ear infections [100,181]. Some undergo an ear tube insertion after childhood. Hearing loss in MPS IVA is progressive [184–186] and bilateral [9, 93] in general, and its severity ranges from mild to moderate [9,18,94] but can be severe [181,185,186]. Permanent hearing loss in MPS IVA is not usually identified until adolescence [176] but has been found in children younger than eight years old [184]. Younger patients exhibited conductive hearing loss and the sensorineural hearing loss element is added to the conductive component as the disease progressed [176,181,184]. A recent study reported that some MPS IVA patients who had normal audiometric results exhibited abnormal auditory neurophysiological responses based on otoacoustic emissions and/or abnormal auditory brainstem responses [181]. Their study suggests that the sensorineural hearing impairment may be in progress before patients notice [93].

Conductive hearing loss can be secondary to recurrent serous otitis media [176] or deformity of the ossicles [62] due to the GAG accumulation in the middle ear [94]. The cause of sensorineural hearing loss is unknown, but it is likely due to the GAG accumulation in the cochlea and retrocochlear auditory nerves [176,187,188].

A recent study [181] on hearing function in patients with MPS IVA showed a strong correlation between height and hearing sensitivity and a strong correlation between height and the cochlear outer hair cell function. The strong relationship between short height and hearing loss suggests that patients with severe skeletal dysplasia may be at higher risk for developing severe hearing loss [181].

In addition to annual behavioral audiometric testing appropriate for the age of patients [176,183,189], regardless of age or skeletal severity, neurophysiological hearing testing such as otoacoustic emissions and ABR should be tested annually to monitor hearing disorders due to the progressive nature of the hearing impairment and the increased risk of developing a sensorineural hearing loss with age [88, 93].

3.4.3 Dental

Deciduous teeth erupt normally and are widely spaced and discolored with thin irregular (stippled) enamel and small pointed cusps which flatten over time with normal wear [54] (Fig. 17). Permanent teeth also have hypoplastic enamel [190]. Some common features have been reported with thin tooth enamel as well as multiple cavities.

Levin et al. described the classic oral abnormalities found among 12 patients with MPS IVA [191]. Tooth morphology is highly specific for MPS IVA [192]. The maxillary anterior teeth are widely spaced and flared, and the posterior teeth are tapered with pointed cusp tips. The enamel can be of normal hardness, but some

patients have pitted enamel with decreased thickness [191]. In roentgenograms, the enamel was less than one-fourth of normal thickness but was of normal radio density [191]. The dental abnormalities in the MPS IVA are of a type that is unique among the group of genetic MPS [191]. The teeth of patients with MPS IVA are typically small, and the enamel is thin with a greyish color. The cusps of the permanent teeth are sharpened [102]. Due to both dental and upper extremity abnormalities, maintaining oral health can be particularly challenging [186].

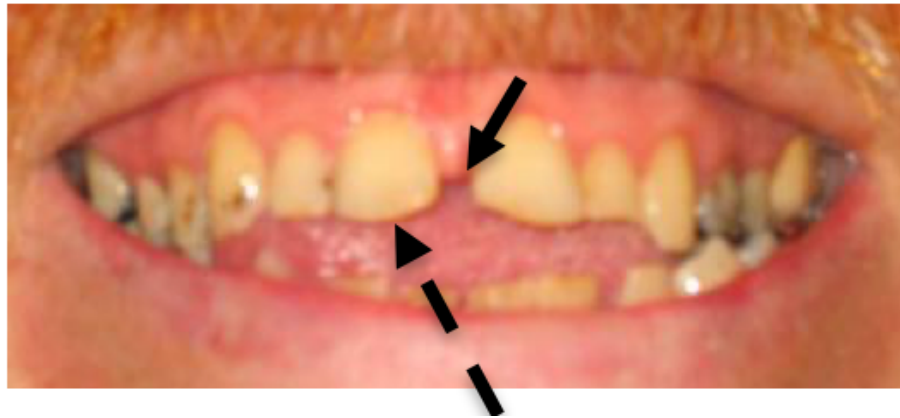


Figure 17: Teeth of a patient with MPS IVA. Image of a 24-year-old patient with widely spaced teeth (black arrow) and spade shaped incisors (black dotted arrow) (adapted from Educational CD for Morquio and permitted by Carol Ann Foundation).

3.4.4 Cardiology

Cardiac complications include ventricular hypertrophy and early onset, severe valvular involvement as well as coronary intimal sclerosis [176]. Cases of cardiac valve thickening, regurgitation, and/or stenosis have been reported in patients [193–197]. In a case study in 1990, 10 patients with MPS IVA underwent echocardiographic assessment, and abnormalities were detected in 6 cases with mitral valve involvement in 5 patients and aortic valve disease in 4 [194]. One patient had severe mitral leaflet thickening to the point of mitral stenosis [194]. Two patients had evidence of myocardial involvement by way of echocardiographic ventricular hypertrophy [194]. Overall, there is a high prevalence of silent cardiac abnormalities in patients with MPS IVA with predominantly left-sided valve involvement [194]. Valve thickening that can be seen on cardiac ultrasound has been found to be nearly as common in patients who do not accumulate dermatan sulfate as in those who do [198]. All MPS IVA cardiac valves may show GAG deposition, although the left-sided cardiac valves are more severely affected [176].

The single cardiac ultrasound study that specifically addresses MPS IVA lacks the color flow Doppler technology which improves the detection of valve stenosis and insufficiency [194]. Despite the absence of color Doppler, valvular heart disease was found to be quite common in the single study devoted to MPS IVA [194]. 5 of the 10 (50%) patients studied had mitral valve regurgitation (1 of whom had mitral valve

stenosis as well), 30% had aortic valve regurgitation, and 20% had both mitral and aortic regurgitation in conjunction with left ventricular hypertrophy [176]. Aortic and/or mitral valve thickening was present in 40% of patients tested [176].

Electrocardiography (ECG) examinations were carried out in 20 out of 37 patients [199], and measurements were compared with normal data [200]. All 20 patients had low R wave voltage in V6 [199]. Among the 37 patients who had echocardiography, cardiovascular abnormalities progressed with age although most had mild clinical signs and symptoms [199].

3.4.5 Respiratory

Respiratory complications are a major cause of morbidity and mortality in MPS IVA. This includes airway obstruction, sleep-disordered breathing, and restrictive lung disease [54]. The GAG accumulation in the following locations: adenoids, tonsils, pharynx, larynx, trachea, and bronchial tree leads to adenotonsillar hypertrophy, tracheal distortion, trachea- and bronchomalacia and obstructive sleep apnea [201,202]. Restrictive lung disease results from a small thorax, chest wall abnormalities, spine deformities, and neuromuscular compromise from cervical myelopathy and hepatomegaly causes an upward displacement of the diaphragm [176,202].

Patients with MPS IVA often prefer to sleep prone on a flat surface without a pillow to keep the neck extended and to minimize the tortuosity of the airway [54].

Due to the short stature and skeletal dysplasia, alteration in growth and development provides an additional mechanism for respiratory compromise which is frequently noted in patients [102]. Patients with MPS IVA are at increased risk for complications that include recurrent infections, progressive loss of respiratory function, sleep-disordered breathing, and ultimately respiratory failure [100,102,203].

The cause of restrictive lung disease in patients with MPS IVA is likely multifactorial; however, thoracic deformity (pectus carinatum, rib deformity) appears to be a primary cause [55]. Pulmonary function tests (PFT) such as forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁) should be performed regularly to assess changes in lung volume and obstruction [55]. Pulmonary hypertension, which may be caused by obstructive sleep apnea, is observed in patients with MPS IVA as commonly as observed in other MPS [55]. Patients may complain of nocturnal dyspnea, in which case polysomnography can be used to assess sleep disturbances [55]. 22 MPS IV patients (7 male, 15 females) ranged from 3 to 40 years of age, underwent non-invasive PFTs with vital signs [204].

For lung function studies, the age, size, and fitness are all important considerations [176]. As patients with MPS IVA exhibit a restrictive and obstructive element of respiratory compromise, both aspects need to be assessed [176]. To assess airflow limitation, the most common test utilized is the spirometry [176]. It is also a readily available technique utilized to measure the vital capacity (VC; the maximum amount of air that can be inhaled/ exhaled from full inflation to maximum deflation)

which indicates the size of the lungs [176]. Airway resistance is assessed through a measurement known as impulse oscillation (IOS), where it utilizes a speaker to impose external oscillating pressure and airflow impulses over these subject's tidal breathing [176]. The "gold standard" assessment of lung restriction is by measurement of total lung capacity (TLC) [176]. This can be assessed by a variety of techniques such as physiological, which includes whole body plethysmography, gas (typically helium) dilution, and nitrogen washout, or by the radiographic means [conventional chest radiographs or (CT)] [176].

3.4.6 Tracheal Obstruction

Unlike other MPS patients, MPS IVA patients develop severe tracheal stenosis with a characteristic appearance on imaging studies and on direct bronchoscopic examination. Thus it is important to remember that MPS IVA patients have obstruction of large airways in addition to the upper airway obstruction reported in the earlier literature. Characteristics of the tracheal abnormality include right ward deviation of the trachea, twisting and buckling of the trachea which may at a younger age appear simply tortuous. Factors that contribute to this unusual pathological features are both external and internal to the trachea itself. External factors consist of the compression of the trachea due to the crossing of the brachiocephalic artery across and anterior to the trachea, proximity of the cervicothoracic spine which moves forward, the sternum (the manubrium) with the clavicular heads, and the pectus carinatum all competing for space in a crowded thoracic inlet, each contributing

varying degrees of compression and narrowing of the trachea [95]. Internal factors contributing to the tracheal pathology include deposits of GAG increasing the thickness of the tracheal walls; unbalanced longitudinal growth of the trachea (in comparison to the growth of the thoracic cavity) resulting in trachea having to ‘fold upon itself’ which is now known as ‘buckling’ of the trachea [55] [83]. Patients with MPS IVA can develop respiratory failure secondary to reduced chest wall compliance and airway collapse due to the irregularly shaped vocal cords and trachea [203]. Tracheal obstruction also leads to life-threatening complications during anesthesia because of the difficulty in managing both upper and the lower airways in MPS IVA primarily manifested by the difficulty in intubating the trachea [83]. Tracheal narrowing increases with age as does anesthetic risk from both upper airway and tracheal pathology[83].

Sagittal MRI images of the cervical spine of 28 MPS IVA patients (12 ± 8.14 years) showed that 19 out of the 28 (67.9%) patients had at least 25% tracheal narrowing. 8 out of 28 (28.6%) patients were categorized as severe (>75%) tracheal narrowing when images were evaluated in neutral head and neck position. The tortuous brachiocephalic artery was the most common cause in the 19 patients with tracheal narrowing ($n = 15$). Evidence of such tracheal narrowing was evident as early as 2 years of age. Tracheal narrowing increased with age, with all 8 patients over 15 years of age having greater than 50% narrowing. The severity score of the subjects over 15 years of age was nearly twice higher than the score of the 10–15 years old

[83]. Greater attention to the trachea is needed when evaluating cervical spine MRIs as well as other imaging and clinical investigations, with the main goal of establishing a timely treatment protocol to reduce the mortality rate in the MPS IVA population.

3.5 Clinical tests

Since MPS is a progressive disorder that affects many organ systems simultaneously, it is very challenging to identify signs and symptoms that can be applied as suitable parameters for clinical trials. Some clinical endpoint tests are performed by measuring the general endurance since the impairment of stamina is common in all forms of MPS. The longitudinal, prospective MPS IVA clinical assessment program reviewed a decline of endurance tests, 6-minute walk test (6-MWT) and 3-minute stair climb test (3-MSCT) suggesting decreased functional ability over time [205]. Other tests can be used as clinical endpoints in studies that are aimed to demonstrate the efficacy of a new drug for MPS patients such as the stair climb test, lung function, joint range of motion, pain, and quality of life.

3.5.1 6 Minute Walk Test (6-MWT)

The 6-minute walk test (6-MWT) is a supervised test that measures the distance a patient can walk on a hard flat surface over a 6-minute period [206]. The criteria of the walking course include a straight, hard, and flat surface, measuring 30 meters (100 ft.) in length with clearly defined turn around points [207]. The walker may go at their own pace, resting as needed, within the prescribed testing time [208]. The 6-MWT is superior to the other walk tests because it is the easiest to perform and

has a good correlation with the functions of the heart and lungs. To evaluate the functional capacity, Balke described a simple test by measuring the distance walked during a defined period [209].

From the 176 patients in the intent-to-treat (ITT) population, 59 patients were randomized to receive placebo, 59 patients to elosulfase alfa 2 mg/kg every other week (QOW), and 58 patients to elosulfase alfa 2 mg/kg weekly (QW) [208]. MOR-004 had a duration treatment up to 24 weeks [208]. Patients who received 2 mg/kg QW demonstrated a mean increase of 37 meters in the distance walk while those who received 2 mg/kg QOW and placebo had a mean increase of 15 meters and 14 meters, respectively from baseline to week 24 [208]. A treatment effect was observed only in the elosulfase alfa 2 mg/kg QW treatment group based on the ANCOVA model [208].

Upon completion of MOR-004, patients rolled over to extension trial MOR-005, the duration which lasted up to week 240 [208]. All patients participating in MOR-004 were eligible to roll over into the long-term efficacy and safety trial MOR-005; however, from the 177 patients randomized into the MOR-004 trial, 173 participated in the MOR-005 trial [208]. Patients who remained on elosulfase alfa 2 mg/kg QW in the extension trial (QW/QW treatment group) achieved a peak increase in mean 6-MWT of 42 meters after a total of 36 weeks of exposure (or week 12 in the MOR-005) [208]. After a total of 48 weeks of exposure (week 24 in the MOR-005), the mean change in 6-MWT for the QW/QW treatment group decreased back to 33 meters [208]. The patients who received placebo during MOR-004 and subsequently

randomized to receive elosulfase alfa 2 mg/kg QW in MOR-005 (PBO-QW) did not experience any improvement in 6-MWT with the initiation of therapy [208].

Overall, treatment with 2 mg/kg elosulfase alfa weekly resulted in a modest improvement in the 6-MWT in the first clinical trial of 6 months; however, there was no improvement in the extension clinical trial (QW-QW) [136]. The 6-MWT has been used widely to assess clinical outcome; it also has its limitation because there is a wide range of variability in how the test is administered despite the availability of guidelines, and the test is effort-dependent [208]. This is particularly problematic in pediatric patients whose performance is often influenced by their developmental stage, understanding of the instruction, willingness to cooperate, training, and motivation (a psychological effect) [208].

3.5.2 3 Minute Stair Climb Test (3-MSCT)

The 6-MWT is a submaximal exercise test widely used to measure endurance and flexibility a range of patients [130,210,211], whereas the 3-minute stair climb test evaluates the effort required by the cardiovascular, pulmonary and/or musculoskeletal systems to perform an activity [211,212]. The 3-MSCT was performed by 274 evaluable subjects which showed a wide range of 0.0-115.0 stairs/min, with a similar mean and median of 30.0 ± 24.0 and 29.0 stairs/min [213]. Although no normative data is available for the 3-MSCT, the stair climb rate of 29 (stairs/min) reported in MPS IVA patients here shows more significant impairment when compared to the baseline stair climb rate of 50 ± 29.5 in the other MPS population such as the MPS VI

population [214]. Overall, there was no significant improvement for patients treated in neither the 2 mg/kg WT nor 2 mg/kg QOW treatment group, compared to the placebo group at week 24. The two treatments groups and the placebo group mean difference in the stair climb rate was only 1.1 stairs per minute [215].

3.6 Pulmonary Function Test

Respiratory function tests are difficult to perform in MPS IVA patients due to their characteristic skeletal dysplasia, small body size and lack of cooperation of young patients, causing conventional spirometry for pulmonary function to be very challenging in some cases [204]. The non-invasive pulmonary tests: impulse oscillometry system (IOS), pneumotachography (PNT), and respiratory inductance plethysmography (RIP) in conjunction with conventional spirometry were evaluated in MPS IVA patients, all subjects had normal vital signs at rest including and age-appropriate heart rate [204]. All patients also preserved normal values in IOS, PNT, RIP, and forced expiratory volume in 1 second/forced expiratory volume total which were normal and not significantly impacted by age; however, the predicted forced expiratory total decreased with age and was below normal [204]. The proposed non-invasive pulmonary function tests can cover a greater number of patients (young patients and/or wheel-chair bound), which provides a new diagnostic approach for the assessment of lung function in MPS IVA that in many cases may be difficult to evaluate [204]. In a natural history study, changes in the FVC and maximum voluntary ventilation (MVV) were observed. For patients less than 14 years, the values

increased, as would be expected for growth; however, the values decreased in older patients, which raised concerns about disease progression [205]. The compromised respiratory function and decreased FVC and MVV are related to multiple factors, including the progression of bone abnormalities and airway obstruction [216]. There were multiple tertiary endpoints explored in the MOR004 clinical trial; however, no clinically important change was seen in the majority of these endpoints by week 24 of the trial [217]. At week 24, both elosulfase alfa treatment groups had mean increases in MVV of 1.5 L/min compared to 0.5 L/min in the PBO treatment group [217]. Due to high variability between patients, the percent change in MVV from baseline to week 24 was used for primary analysis [217].

In children and adolescents, lung volumes can lag behind standing height growth rates [217]. No standards exist to reliably estimate predicted pulmonary function values in the MPS IVA population due to their early growth arrest and short stature [217]. However, pulmonary function tests (FVC, FEV₁, MVV) were conducted in relation to the primary endpoint at the MOR004 [217]. The results of the correlation analyses of baseline PFTs and the change from baseline to week 24 in 6-MWT distance revealed weak relationships between these parameters [217]. Continued treatment with elosulfase alfa for an additional 24 weeks led to further improvement in MVV in the QOW-QOW group but not the QW-QW group [217].

3.7 Range of Motion

Patients typically show significant differences in the active and passive range of motion (ROM) at the wrist joint, which shows the loss of stability at the joint [218].

Patients typically show a pronounced decrease in grip and pinch strength and have difficulties performing day-to-day tasks that require strength [218]. This regular assessment of ROM and strength provides insight into the degree and progression of functional impairment of the hands [164]. A goniometer can show both active and passive ROM of the wrists and digits [164].

3.8 Sleep Study

Sleep-disordered breathing (SDB) is common in all MPS diseases and may precede the development of overt respiratory failure during wakefulness [176,201].

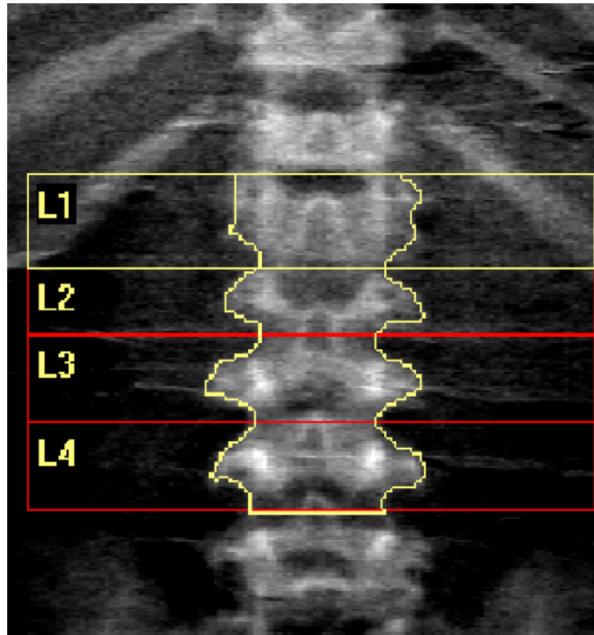
Ventilatory abnormalities during sleep include obstructive sleep apnea due to several factors such as GAG accumulation in the upper airway, sustained hypoventilation due to the chest wall deformity and/or respiratory muscle weakness [176]. Sleep studies should also be performed in addition to lung function assessments [176]. Symptoms suggestive of SDB, such as apneas, gasping respirations, snoring, difficult wakening, daytime somnolence, and restless sleep should be assessed annually as part of the clinical evaluation of all patients [176]. Overnight sleep studies can be used to both diagnose the type and severity of SDB and also to evaluate nocturnal ventilatory treatments for the underlying respiratory disorder [176].

3.9 Bone Mineral Density

In the last few years, MPS IVA has been reported to be related with an early-onset osteoporotic phenotype, which can affect the clinical course of the condition [103]. The accumulation of KS disturbing bone mass acquisition and perturbing the regular microarchitecture of bone tissue has been reported to reflect on the bone mass density (BMD) [219]. Patients with MPS have an increased risk of poor bone mineralization due to malnutrition, a particularly small frame, and abnormal gait, and reduction of physical activities is caused by pain, poor health condition, or exercise intolerance [220]. Bone growth and mineralization have been reported to be affected by GAGs accumulation in animal models of MPS; there are, however, limited publications on the assessment of bone mineral density in patients with MPS [127,220–223].

In a study with 18 patients (16 unrelated) with MPS IVA with an average age of 21.4 years (3.3 to 40.8 years). In this prospective cross-sectional study, BMD of the whole body (WB), lumbar spine (LS), and lateral distal femur (LDF) was acquired by dual-energy X-ray absorptiometry (DXA) on patients with MPS IVA (Fig. 18). WB DXA could be obtained on only 6 patients (5 full-time ambulators) because of the respiratory compromise caused by the position, presence of hardware, or positioning difficulties. Mean WB Z-score was -2.0 (range -0.3 to -4.1). Technical issues invalidating LS DXA in 8 patients included kyphosis at the thoracolumbar junction results in overlap of vertebrae in the posterior-anterior view. Mean LS BMD Z-score in full-time ambulators was -3.4 (range -1.6 to -5.0) and in the non-/partial

ambulator was -4.0 (-3.7 to -4.2). Lateral distal femur BMD was acquired on every patient, and average Z-scores were -2 or less at all the sites; fulltime ambulators exhibited higher BMD. LDF proved to be the most feasible site to measure in patients with MPS IV A [224]. Low BMD of the lower extremities as measured by the LDF, DXA is directly associated with lack of ambulation or weight bearing [225–229].



DXA Results Summary:

Region	Area (cm ²)	BMC (g)	BMD (g/cm ²)	T - score	Z - score
L1	6.34	2.00	0.315		
L2	4.49	1.76	0.392		
L3	5.81	2.67	0.459		
L4	6.22	3.27	0.525		
Total	22.87	9.69	0.424		-1.6

Total BMD CV 1.0%

Figure 18: DXA image of the spine of a MPS IVA patient. Lumbar spine DXA requires careful and precise measurement of vertebral body levels and margins (Adapted from Kecskemethy et al. Mol Genet Metab; 2017, 144-149) [224].

3.10 Dexterity Test

The Functional Dexterity Test (FDT) is a validated and timed pegboard test that is not only easy to administer, but also it assesses the ability of the patients to perform functional daily tasks that require a 3-jaw chuck pretension pattern (also referred to as the lamar pinch, pencil pinch or tripod grip) such as writing and buttoning [230,231]. Each patient with MPS IVA syndrome requires regular assessment of upper limb function (fine motor skills) [166]. To obtain consistent measurements, it is important to record the position of the wrist and also to record whether the wrist was supported or not during testing [166]. Passive and active assessments of the range of motion of elbows and shoulders can be useful but do not have therapeutic consequences [166]. The FDT was developed as a measure of dexterity that will take a minimum amount of time to administer, yet still provides information regarding the patient's ability to use the hand for daily tasks requiring a 3-jaw pretension between the fingers and the thumb [231]. The overall, recommended pediatric modifications to the FDT are to use speed (pegs per second) instead of time (seconds) to report the results, and also to not assess penalties [230].

3.11 Gait pattern

Tests on gait and mobility can easily be done by a simple physical exam and an interrogation of the patients [166]. Instrumented gait analysis provides information about the dynamic alignment of the lower extremities and is also helpful in decision making in various conditions [232]. In a study of 9 MPS IV children (who had no previous lower extremity surgery), underwent a 3D gait analysis to describe the gait

kinetics and kinematics in children with MPS IV. The mean age at gait analysis for the study was 10.6 ± 4 years, mean height was 105.2 ± 15.6 cm ($z = -4.5$), and mean weight was 22.3 ± 7.2 kg ($z = -1.434$). The measurements obtained from gait analysis in the patients were compared with data from 10 healthy young individuals with age ranging from 9.5 to 11.5 years; the mean height was of 138 ± 6.6 cm, and the mean weight was of 32.5 ± 7.1 kg [163]. There were significant differences in the temporal-spatial characteristics, kinetics, and kinematics in children with MPS IV in comparison with the normal population on instrumented gait analysis. There was a decreased forward velocity, cadence, and stride length that was compared with the normal population ($p < 0.05$). The height-adjusted, normalized forward velocity and stride length were also reduced in comparison with the normal population ($p < 0.01$). The forward tilt of the trunk and pelvis, hip flexion, hip adduction, and external hip rotation were increased in comparison with the normal population ($p < 0.05$). There was increased knee flexion, genu valgus (abduction), and external tibial torsion compared with normal during stance ($p < 0.05$). Dynamic knee varus–valgus joint laxity showed a mean difference of 9.5° between the minimum and maximum genu valgus (abduction) that was recorded during stance, which is compared with a difference of 0.9° in the normal population ($p < 0.05$). While there was a strong correlation between genu valgus measured on gait analysis and standing radiographs ($r = 0.89$), there was a moderate correlation between genu valgus measured on gait analysis and clinical examination ($r = 0.69$) [163].

3.12 Activity of daily living

The current options for treating MPS IVA in a multisystem manner to address overall quality of life are limited [176]. Currently, due to the absence of an effective and safe systemic treatment option, patients require extensive management and regular intervention to maximize their quality of life [176]. To track and manage the quality of life, patients with MPS IVA should see physiotherapist annually to evaluate the degree of impairment, including determination of the range of motion, muscle strength, daily activity, and participation limitations [176]. As MPS IVA disorder process progresses, the quality of life for the patient declines. Patients increasingly become more dependent on caregivers as deteriorating vision, hearing, oral health, respiratory and cardiac function, muscular strength, and endurance make routine daily activities increasingly difficult to complete tasks [176].

The activity of daily living (ADL) questionnaire is made up of three sections: “Movement,” “Movement with Cognition,” and “Cognition.” Each section has four subcategories rated on a 5-point scale based on the level of assistance. The questionnaire was then collected from 145 healthy controls and 82 patients with MPS IVA. Of the 82 patients questioned, 63 patients were severe and 17 patients had attenuated phenotypes (2 were undefined); 4 patients treated with hematopoietic stem cell transplantation (HSCT), 33 patients with enzyme replacement therapy (ERT) for more than one year and 45 untreated patients. MPS IVA patients showed a decline in ADL scored after 10 years of age. Patients with a severe phenotype had a lower ADL score than healthy control subjects, and lower scores than patients with an attenuated

phenotype in domains of “Movement” and “Movement with cognition.” Patients who underwent HSCT were followed up for over 10 years, had higher ADL scores and fewer surgical interventions than untreated patients [233].

3.13 Growth

Growth impairment is commonly observed in patients with MPS [234].

Patients with MPS IVA are most severely affected in growth and final height, which are used as an indicator of disease severity [100,235]. Short stature is the main evident symptom, and the male and female patients have the growth impairment to approximately an equal degree [100,213,235]. Growth in patients with MPS IVA stops around 7 to 8 years age [235,236].

In 2007, Montano et al. described that the phenotypic classification for severe MPS IVA by height was below 120 cm while the classification for the attenuated type was above 120 cm [236]. Some patients with attenuated MPS IVA continue growing even into their teens and reach over 140 cm [100]. In 2008, Montano et al. proposed that a standard growth chart for each gender of MPS IVA patients should be made for better results of phenotypic classification [236]. Height and weight measurements from MPS IVA patients were collected in the International MPS IVA Registry, and the growth charts for male and female patients were established, compared to reference growth curves of healthy children provided by the CDC [55,100]. In 2012, more height and weight measurements from 193 girls and 195 boys with MPS IVA were collected to revise the growth charts [147]. The mean birth

length of boys was 52.4 ± 3.9 cm, and the mean height for males at 18 years of age was 119.3 ± 22.6 . The mean birth length of girls was 52.1 ± 2.9 cm, and the mean height for girls at 18 years of age was 113.5 ± 23.1 cm. These values correspond to -8.0 SD and -7.7 SD of the mean height for normal males and females. The mean birth weights for boys were 3.56 ± 0.5 and for girls were 3.5 ± 0.7 kg. The growth patterns in MPS IVA patients were characterized by impaired growth velocity after 1 and 2 years of age [235]. According to the isopleth upon which the patient falls, patients above the 90th centile on the growth chart for each gender of MPS IVA are more likely to be defined as mild attenuated while patients between the 75th centile and 90th centile on the growth chart are more likely to be defined as intermediate attenuated. Patients less than the 75th centile are classified as classic severe.

In another study with MPS IVA from Taiwan, 92% of the patients had short stature [237]. Patients treated with ERT showed no statistical significance in height/growth rate [215,238]. Overall, the height and growth velocity are the simplest and objective tests to define the clinical severity and to monitor therapeutic efficacy.

3.14 Molecular Diagnosis

Molecular analysis is used to confirm the diagnosis and to provide genetic counseling for the family and prenatal analysis [101]. The *GALNS* gene is located on chromosome 16q24.3, contains 14 exons, and generates a 1566 nucleotide mRNA [239–242]. Multiple MPS IVA mutations are believed to be exclusive and found in only one individual or family [243].

As of February of 2018, 334 mutations in the *GALNS* gene have been reported missense/nonsense mutations account for a total of 203, 35 deletions, 22 splicing site mutations, 7 insertions and 3 complex rearrangements [244]. Several mutations are common; the most prevalent recurrent mutations in the *GALNS* gene are c.1156C>T (p.R386C), c.901G>T (p.G301C), c.337A>T (p.I113F), c.1A>G (p.M1V), c.757C>T (p.R253W), c.871G>A (p.A291 T), c.935C>G (p.T312S), and c.1171A>G (p.M391V), accounting for 8.9%, 6.8%, 5.7%, 2.3%, 2.1%, 1.8%, 1.8%, 1.8%, and 1.8%, respectively [245–251].

3.15 Conclusion

Variable clinical presentation and laboratory testing caveats make MPS IVA particularly challenging to diagnose [96]. Both skeletal and non-skeletal symptoms need to be tested and assessed for clinical suspicion. Especially, imbalance of growth causes major clinical signs and symptoms leading to serious morbidity and mortality. Radiographic, biochemical, and molecular tests are critical for precise diagnosis and prognosis for MPS IVA. The age of diagnosis, the natural progression of the disease, and prognosis of the disease play a role in determining the precise management of MPS IVA.

Chapter 4

DIAGNOSIS OF MPS

4.1 Urinary and Blood GAG

Analysis of levels of undegraded GAGs in urine is generally used for the first approach to diagnosing MPS [252]. The measurement of total GAG excretion in urine is widely used as a biomarker for MPS [253–255]. Total urine GAGs can be performed quantitatively and qualitatively [76]. Patients with MPS have a gross elevated urinary GAG level, whereas some patients can have borderline or even slightly elevated urinary GAGs, especially patients with MPS IV, which results in false negatives in a screen [104–107,111,145]. False negative results are found for almost 15% of all MPS patients [106] and even more for MPS IV patients [104–106]. The urinary excretion of GAGs is high in infants and young children, decreases with age, plateaus by the second decade of life, and remains constant through adulthood [105,106,108–110].

The qualitative analysis of total urinary GAGs is usually performed by spectrophotometric analysis using 1,9-dimethylmethylene blue (DMMB), to detect GAGs in urine from older children [108,111,256]. In 1989, Whitley et al. showed that the DMMB testing could be used as potential screen for any MPS disorder because the test measures levels of all GAG species on the urine spot [96,105]. Currently, DMMB

has been used for diagnosis and for determining therapies in clinical trials for MPS I, II and VI [257–259]. DMMB works best with isolated GAGs or urine sample, but can be adapted to tissue samples as well [260]. For qualitative urine-based testing methods, the GAGs are first isolated from the urine and then separated by thin layer chromatography or electrophoresis [112–115]. KS can be difficult to separate from the age-matched controls by these methods, and false negatives can easily occur [261]. DMB assay is able to detect high molecular mass GAGs along with fragments [111,262], whereas the agarose-gel electrophoresis is capable of elevating molecules that have a molecular mass greater than 1500 [263,264].

Dye-spectrometric methods such as alcian blue [265] and DMMB [105,108,111,266–268] have been used to assay total urinary GAG. Another method known as the thin-layer chromatography (TLC) has been used to separate specific GAGs, however TLC cannot measure GAGs in blood or tissues [82]. Dye-spectrometric and TLC methods are not sufficient to detect all types of MPS, especially MPS IV [82]. TLC is quick and inexpensive, however it provides poor quantification and accuracy [269].

4.2 MPS and GAGs

Each subset of MPS have specific derived polysaccharides that affect that individualized MPS. MPS I and II have heparan and dermatan sulfate derived polysaccharides that are excreted in the urine, whereas MPS III excretes only heparan sulfate derived polysaccharides and MPS IV patients have an accumulation of keratan

sulfate that is excreted in the urine. MPS VI excretes only dermatan sulfate derived polysaccharides. Patients with MPS VII have a combination of dermatan, heparan and chondroitin sulfate that can also be detected in the urine [252].

Keratan sulfate (KS), is a linear polymer GAG, composed of alternating D-galactose (Gal) and N-acetyl-D-glucosamine (GlcNAc) [270]. Keratan sulfate proteoglycans have been found in the cornea, cartilage, and bones [271]. KS is involved in cell motility, embryo implantation, wound healing, corneal transparency, extracellular matrix of cartilage, and neuronal regeneration [272]. Deficiency of one of the enzymes involved in the process of degradation of KS, results in storage of partially degraded KS, causing both cellular and organ dysfunction [273]. KS is mostly synthesized and accumulated in the cartilage and cornea in MPS IVA patients, and excessive accumulation of KS in lysosome leads to disruption of chondrocytes and subsequent increase of KS levels in blood and urine [70].

Di-sulfated KS is dominant in shark cartilage and rat serum, whereas mono-sulfated KS is dominant in bovine cornea and human serum [270]. Levels of mono- and di-sulfated KS varied with age in blood and urine from control subjects as well as patients with MPS II and MPS IVA.

The detection of KS in biological specimens with tandem mass spectrometry (MS/MS) was first developed by Oguma et al. [118] and applied to specimens in MPS

IVA patients. Oguma et al. was able to distinguish between control subjects and MPS IVA patients without false negatives [119–121].

KS is elevated in blood from several types of MPS, even when KS is not the primary storage GAG [76]. In patients with MPS IVA, KS levels in blood and urine are associated with age and clinical severity [120]. According to Shimada et al., the mean levels of both forms of KS in the plasma/serum from patients with MPS II, IVA, and IVB were elevated compared with age-matched controls. Blood KS level was elevated in patients with MPS II and MPS IVB [120,145].

MPS IVA and IVB patients have a deficiency of the enzyme that is directly involved with KS metabolism, causing an elevation of KS in the blood and urine for this type of MPS to be expected; MPS IVA (GALNS) and MPS IVB (GLB1). Blood and urine KS levels are higher in MPS IVA patients, compared with age-matched controls [12, 37–39]. Urine KS levels remain higher or subnormal in MPS IVA patients than those in controls after 20 years of age; however, blood KS levels tend to be normalized by the age of 20 years [119–121]. However, patients with other types of MPS, where the respective enzymes do not directly involve the pathway of KS, also have elevation of KS in the blood and urine as well [270]. There is significant overlap of KS levels between patients with MPS IVA and age-matched controls, especially in patients older than 10 years. This suggests that better biomarkers for MPS IVA are needed [120].

Heparan sulfate (HS) is involved in diverse biological functions including activation of growth factors [275]. HS GAG chains are linear polysaccharides composed of alternating N-acetylated or N-sulfated glucosamine units (N-acetylglucosamine (GlcNAc) or N-sulfoglucosamine (GlcNS) and uronic acids (GlcA or IdoA) [275]. HS derived disaccharides are sensitive markers for NBS for MPS I, II, and III [252]. MPS I, II, III, and VII patients cannot properly degrade HS. A sandwich ELISA method for HS measurement in MPS patients showed that blood and urine HS levels are elevated in MPS I, II, III, VI, and VII and correlate with clinical severity [80].

The detection of KS in biological specimens with tandem mass spectrometry (MS/MS) has been developed first by Oguma et al. [118] and applied to specimens in MPS IVA patients and distinguished between control subjects and MPS IVA patients without the false negatives [119–121]. Blood and urine KS levels are higher in MPS IVA patients, compared with age-matched controls [12, 37–39]. Urine KS levels remain higher or subnormal in MPS IVA patients than those in controls after 20 years of age; however, blood KS levels tend to be normalized by the age of 20 years [119–121].

Using sandwich ELISA [104,122] and LC/MS/MS [118,120] assay, the study was conducted for KS levels in blood and urine from MPS IVA patients and healthy controls to evaluate the comparability of results.

The levels of blood and urine KS correlate with clinical severity during the early and progressive stage of the disease, and therefore, it is a good prognostic biomarker at this stage [98]. Blood KS directly displays growth, turnover disruption, and/or repair of cartilage where it is mainly synthesized [98]. The advantage of measurement of KS in dried blood spot (DBS) testing is its convenience for transport of samples and screening purposes [98]. In contrast, urine KS has a broader range of value and may not reflect cartilage condition directly. The origin of urine KS derives directly from the kidney, and urine KS is filtered in kidney. Therefore, only selected smaller molecules are excreted in urine [98]. When MPS IVA patients are treated with ERT, it is likely that urine KS levels are reduced rapidly since the enzyme is delivered to kidney and digests the KS stored in the kidney [129]. Thus, urinary KS is useful to differentiate MPS IVA from other forms of MPS and to demonstrate pharmacodynamic effects of therapy while it does not provide a valuable predictor (biomarker) of skeletal or clinical improvement during these therapies for MPS IVA [130]. It is noteworthy that the origin and character of urinary GAGs are different from those of blood GAGs as seen is KS [131].

GALNS plays a role as galactose-6-sulfatase (G6S) because the enzyme hydrolyzes the sulfated galactose of KS and converts di-sulfated KS to mono-sulfated KS [130]. Therefore, deficiency of GALNS activity leads to the accumulation of more di-sulfated KS, and therefore, the ratio of di-sulfated KS to total KS of patients with MPS IVA increases, compared with normal controls especially at an early stage [130]. Levels of both mono-sulfated and di-sulfated KS in blood were measured by

LC/MS/MS, and patients with MPS IVA had higher KS with both forms, compared with age-matched controls [134]. The elevation of di-sulfated KS in MPS IVA patients was more significant than that of mono-sulfated KS [130]. The proportion of di-sulfated KS vs. total KS in blood rose with age in control subjects while it was age-independent in patients with MPS IVA. The proportion of di-sulfated KS is better at distinguishing younger MPS IVA patients than older patients from age-matched controls. Levels of mono- and di-sulfated KS in the urine of MPS IVA patients were also higher when compared to those in age-matched controls for all studied ages. A significant difference in sulfation levels of KS between control subjects and patients with MPS IVA indicates that di-sulfated KS is another potential biomarker for MPS IVA [130].

Most MPS patients who lack enzymes required for catabolism of KS or HS have an elevation of KS or HS in blood and urine respectively [88]. More importantly, majority of these patients also had a secondary elevation of KS or HS, regardless of the normal levels of enzymes required to digest the particular GAGs [88]. Tomatsu et al. proved that MPS I, II, III and VI had increased levels of HS and DS derived disaccharides in plasma [120,276]. This was measured by LC-MS/MS which then suggested that this technique might be used for NBS for MPS I [276].

Overall, despite the fact that DS and HS are primary storage materials in most MPS, there is limited understanding in affected patients with regard to when these specific GAG's are elevated in circulation and how they are correlated with the

clinical signs [276]. Determination of urine KS concentration provides a potential biomarker to screen for a high risk of MPS IVA patients and to measure pharmacodynamics effects [76]. This is important for assessing the clinical status at the initial progressive stage. Blood KS could be used for 1st tier newborn screening followed by the enzyme assay or vice versa [98] and is a potential biomarker to provide the clinical severity and therapeutic effect for the bone at the initial progressive stage.

4.3 Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) is a technique based on the binding of an antigen to an antibody that is linked to an enzyme and detection by hydrolysis of a substrate to the linked enzymes [277]. There are four different types of ELISA, direct, indirect, sandwich and competitive. ELISA assays that are developed for GAGs specifically can measure levels of KS [104,145,278], HS [80], C4S [279], C6S [279], DS [279] and hyaluronic acid [280].

The measurement of blood KS was first accomplished by an inhibition ELISA by Thonar et al. in 1988 [281]. Thonar and his colleagues showed that blood KS is age-dependent [281]. Tomatsu et al. developed a sandwich ELISA method for KS measurements in 2004, showing that KS levels in blood and urine of MPS IVA patients are markedly elevated [104,145]. Tomatsu also showed that clinical severity correlates with the levels of blood and urine KS [104]. Tomatsu et al. then proved that

blood KS is elevated in other types of MPS in 2005, proving that the potential use of blood KS for screening and monitoring other types of MPS, besides MPS IV.

The results of ELISA measuring of blood and urine KS and HS measurements are used a biomarker to assess clinical severity at an early stage and to also monitor therapeutic effects [276]. ELISA protocols have been developed to detect GAGs in cells as well as on cell-surfaces [282,283]. It is expected that patients with null or very low enzyme levels will have a severe phenotype, while moderate to higher levels of the residual enzyme will give rise to attenuated phenotype [284].

ELISA assays for CS, DS, KS, and HS in blood and urine have been established that are rapid and reproducible but expensive [82]. It is important to note that to measure KS, HS, or DS by ELISA is not available commercially [76]. The use of ELISA is advantage due to its feasibility, sensitivity, reproducibility and quantitation which only requires a simple ELISA plate reader [82]. However the cost of ELISA is large, since no current assay can detect several GAGs simulation, which requires the use of multiple assays [82].

4.4 Newborn Screening

Newborn screening (NBS) provides early diagnosis for patients with LSDs, allowing genetic diseases to be treated is an imperative preventive disease manifestation. The first NBS was developed by Dr. Robert Guthrie in the early 1960s, in which he developed an assay for phenylketonuria, an inborn error of amino acid

metabolism [285,286]. Since then, NBS has become an important public health program because early intervention can prevent premature mortality, morbidity and numerous disabilities [287]. In the 1990s, tandem mass spectrometry (MS/MS) was adapted for NBS, which allowed the rapid as well as the analysis of amino acid and acylcarnitine profiles, which facilitates the identification of more than 30 different inborn errors of amino acid, fatty acid, and organic acid metabolism for a 3-mm dried blood spot (DBS) sample [288,289]. Further modifications have been made to the MS/MS method to streamline the sample preparation steps, which includes an online separation of analytes by liquid chromatography (LC) coupled to the tandem mass spectrometer (LC-MS/MS) which were developed by Gelb [290] and Kasper [287,291].

NBS is now considered public health process that can enable early identification and treatment for inherited diseases that can positively affect the long-term health of a patient's life [76]. Currently, several states including Illinois, Kentucky, Michigan, Missouri, New Jersey, New Mexico, New York, Ohio, Pennsylvania and Tennessee mandate LSD screening in all newborns [292]. Due to the increase in availability of treatments and the importance of early intervention have stimulated NBS to diagnose LSDs and permit early intervention to prevent irreversible impairment or severe disability [293].

Chapter 5

BIOMARKERS

Disease biomarkers provide clinicians with the tools necessary for early diagnose, accurate prognostication, optimization of therapy, and monitoring of therapeutic responsiveness [294]. Few biomarkers exist to monitor the severity of bone and joint involvement in MPS, or the effects of therapies [295]. In a morphologic analysis of MPS, the growth plates showed clusters of enlarged, GAG-containing cells that disrupted the normal columnar structure of the growth plate cartilage, leading to bone growth abnormalities [296,297]. Since GAGs accumulate within the lysosomes, including the cells of the immune system, it is observed that altered immune responses can be noticed in LSDs [298]. These macromolecules have pro- and anti-inflammatory properties-playing a role as co-receptors for some cytokines [299]. Due to this, there is evidence for the involvement of inflammation in MPS, representing possible molecular biomarkers for MPS [33,36,300,301].

Previous studies have proved that increased apoptosis of MPS chondrocytes lead to a depletion of proteoglycans and total collagen in the cartilage of MPS animals [301]. Patients with MPS secrete pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β), nitric oxide (NO) production, apoptosis of chondrocytes, and reduced proteoglycan content in the ECM [295]. The precise regulation of MMP activity is crucial for maintain the proper balance of tissue

remodeling and damage [295]. Cytokines, such as IL-1 β and TNF- α are produced by MPS chondrocytes, synoviocytes, and macrophages, and significantly up-regulate MMP gene expression [295]. The increased levels of circulating cytokines or other inflammation-based markers could potentially reflect more broadly upon MPS disease.

5.1 Cytokines and Chemokines

Cytokines are small, nonstructural soluble proteins, that are secreted by cells to regulate cellular immunity and the inflammatory response of other cells [302,303]. Cytokines have a molecular weight ranging from 8 to 40,000 d [304]. Cytokines are regulators of host responses to infection, immune responses, inflammation and trauma [304]. Pro-inflammatory cytokines promote inflammation, whereas anti-inflammatory cytokines reduce inflammation, promotes healing, and suppress the activity of pro-inflammatory cytokines [304]. Presently there are 18 cytokines with the name interleukin (IL) [304].

IL-1 and TNF are pro-inflammatory cytokines and can cause fever, inflammation, tissue destruction, shock and even death when administered to human [304]. IL-1 and TNF are inducers of endothelial adhesion molecules which are essential for the adhesion of leukocytes of the endothelial surface prior to the emigration into the tissues [304]. IL-1b, IL-6, and TNF- α are pro-inflammatory cytokines which are induced commonly together. These cytokines are produced peripherally and in the CNS, usually by the microglia. IL-1 affects nearly every cell type, often in concert with another pro-inflammatory cytokine, TNF [305]. TNF- α

stimulates vagal afferents and is produced by neurons and glial cells in an activity-dependent manner. IL-6 and IL-1 are produced in the brain, where they mediate neuroinflammation and response to injury. IL-10 are potent anti-inflammatory agents and are activators of B lymphocytes [304].

IL-6 was discovered in the 1980s as a lymphocyte-derived signal for B-cell maturation which functions both as a pro-and anti-inflammatory cytokine, and has been implicated in a variety of inflammatory and autoimmune disorders [306–308]. IL-6 is a 184 amino acid cytokine produced by both immune cells (macrophages, B cells and T cells, and non-immune cells, endothelial cells and fibroblasts) in response to homeostatic disturbances such as infection and injury [306,309]

Another class of genes that are also pro-inflammatory are chemokines. Chemokines are small peptides, which facilitates the passage of leukocytes from the circulation into the tissues. IL-1 and TNF are inducers of endothelial adhesion molecules. This is essential for the adhesion of leukocytes to the endothelial surface prior to emigration into the tissues. Chemokines are any class of cytokines with functions that include attracting white blood cells to sites of infection. Macrophage inflammatory protein 1 alpha (MIP-1a) is an example of inflammatory chemokine. Taken together, pro-inflammatory cytokine-mediated inflammation is a cascade of gene products usually not produced in healthy people. IL-1 and TNF are effective in stimulating the expression of these genes, whereas anti-inflammatory cytokines such as IL-10 block this process or suppress the intensity of the cascade [304].

MPS I, II, and IVA patients have demonstrated alterations in parameters of oxidative stress and inflammation. Filippon et al. investigated the effect of ERT on several biomarkers of oxidative stress of MPS II. Oxidative stress can play an important role in the pathophysiology of some IEM. Oxidative stress and MPS linkage were performed in animal models [310]. In a study by Villani et al., showed presence of oxidative stress even in early stages of MPS IIIB and oxidative imbalance in an animal model of MPS I.

5.2 Epidermal Growth Factor

Epidermal growth factor (EGF) influences expression of certain genes by binding to its transmembrane receptor that upon interaction becomes an active protein kinase, initiating a specific kinase cascade that finally results in regulation of activity of particular transcription factors [311]. This tyrosine-specific protein kinase activity of the EGF receptor is inhibited by genistein [312,313]. Genistein is used as medicine for treatment of MPS patients.

5.3 Macrophage Inflammatory Protein 1 alpha

Macrophage inflammatory protein 1 alpha (MIP-1a) is an inflammatory chemokine playing an important role in macrophage recruitment, inducing monocytes to infiltrate the CNS and expanding the activated macrophage-microglial subpopulation [314].

5.4 TNF- α

TNF- α is a potential therapeutic target and is involved in a variety of inflammatory pathways that have destructive results such as increased endothelial permeability, inflammatory cell migration and MMPs [315]. The inhibition of TNF- α improves physical function and skeletal disease in animal models of MPS [316]. The mechanism of elevated TNF- α levels in MPS is related to excess of GAGs that stimulate macrophage via toll-like receptor 4 (TLR-4) [317]. Decreasing inflammation through treatment of MPS animals with anti-TNF- α medications or another anti-inflammatory medication, has demonstrated improvements in mobility and exercise tolerance, resolution of joint inflammatory changes, and increased bone length [317–320].

5.5 Metalloproteinase

The stimulation of MPS connective tissue cells by the inflammatory cytokines causes enhanced secretion of several matrix-degrading metalloproteinases (MMPs) [295]. MMPs are pro-inflammatory cytokines, key enzymes involved in ECM degradation and cartilage destruction, and MMP transcription is regulated by growth factors and cytokines, while specific tissue inhibitors of metalloproteinase (TIMPs) regulate their translation and proenzyme activation [321,322]. MMPs are zinc-dependent endopeptidases, classified into collagenases and have been implicated into various processes, both normal and pathological, however usually related to inflammation and cell apoptosis [323–325]. NO production has also been shown to act as a pro-inflammatory mediator leading to enhanced MMP production [326]. An

important feature of MPS is that altered MMP expression is most likely stimulated by inflammatory cytokines and NO [295]. MMPs are secreted from cells as inactive zymogens, and the pro-forms are activated by serine proteinases and other classes of MMPs [295]. MMPs seem to be involved in numerous neuro-inflammatory diseases, and an overproduction of these enzymes are coupled with a disruption of BBB [327], MMP-2 and MMP-9 are a suitable biomarker demonstrating CNS involvement [328].

5.6 PIIANP; Type IIA collagen N-Propeptide

Type IIA Collagen N propeptide (PIIANP), which is a marker for collagen type II formation, was assessed in the serum of MPS IVA patients in a study by Lorget et al. A marked increase in mean PIIANP levels was observed in comparison to baseline levels at week 25, 357, and 72. Resting and proliferating chondrocytes are the main source of collagen type II [329]. Collagen type II is a molecular marker of hyaline cartilage phenotype, since it's the main constituent among cartilage collagens [330].

Chapter 6

TREATMENT FOR MPS

Narrowing the gap between symptoms onset and the diagnosis of MPS is extremely important and relies majority on increased recognition of clinical red flags by the physicians and specialists that manage the MPS related symptoms [43]. The age of diagnosis has not decreased for MPS I phenotypes, which is why the interval between symptom onset and treatment initiation which remains substantial for patients with any type of MPS I.

Currently, enzyme replacement therapy (ERT), hematopoietic stem cell transplantation (HSCT), substrate reduction therapy (SRT), and gene therapy are clinically available or under clinical trials for patients with MPS which leads to the partial restoration of the enzyme activity or inhibition of GAG synthesis [79]. Initiating these treatments for MPS at birth or at a very early stage in the patient's life can provide the greatest benefit for the clinical course of the disease [200–207]. To improve activities of daily living (ADL) for patients with MPS, it is very critical that patients have early diagnosis and early treatment as well.

6.1 Enzyme Replacement Therapy

Treating MPS with ERT relies on the cellular uptake of the enzyme by receptor-mediated endocytosis. ERT is FDA approved and has met with great success

for treatment of non-neurological manifestations for use in patients with MPS I (Aldurazyme) [208], MPS II (Elaprase) [208,209], and MPS VI (Naglazyme) [210,211]. To date, ERT has been approved for patients with MPS I and VI and clinical trials have been initiated for MPS II [2]. Patients treated with ERT have improvement in somatic manifestation and better quality of life.

ERT is a lifelong therapy, that involves intravenous infusions of the recombinant human enzyme either weekly or every other week [212]. Conventional ERT depends on transport of exogenous recombinant enzyme via mannose-6-phosphate/insulin-like growth factor II (M6R/IGFR) or C-type mannose receptors on cells [92].

Enzyme Replacement Therapy (ERT) with laronidase (recombinant human α -L-iduronidase; Aldurazyme, BioMarin Pharmaceutical and Genzyme, a Sanofi Company) was approved in 2003 to treat non-neurological manifestations of MPS I and is the primary treatment option for patients with Hurler-Scheie and Scheie syndromes [43]. Laronidase is also used to treat Hurler patients who are not candidates for HSCT due to age, health status, access to transplant or parental choice [43]. Laronidase must be given as a weekly peripheral or central intravenous infusion and is a lifelong therapy [43]. Laronidase may also be more beneficial when started early, as suggested by a case report of a sib pair with Hurler-Scheie syndrome [213]. Developmental outcomes are better when transplant occurs before 24 months of age

[214]. The timing of treatment initiation and diagnosis is an important factor for the success of HSCT and Laronidase [43].

Recombinant human idursulfase (Elaprase, Shire, Lexington, MA) is approved as ERT for patients with MPS II, has been commercially available since 2006 based on the results of a phase 2 and 4 randomized, double-blind, placebo controlled clinical trial in 96 patients with MPS II, with an age range of 5-53 years of age [117,215–217]. ERT should be offered to all patients with attenuated MPS II older than 5 years of age [218,219]. According to a study by Jurecka et al., early intervention of ERT can slow down or prevent irreversible manifestations of MPS II, including coarse facial features, joint disease, and cardiac function [220]. Benefits in idursulfase that was noticed was improvement in walking capacity, decreased liver and spleen volume and reduction, but not normalization, of urinary GAG levels [117].

Recombinant form of human arylsulphatase B, known as galsufase (Biomarin, Novato, CA, USA) has been available since 2005 [212]. The benefits in galsufase that were noticed are improvements in walking and stair climbing capacity, pulmonary benefit, improvement in growth and reduction of urinary GAG excretion [118,210,211,221–224]. A control study showed that early intervention with galsufase in infancy improved the development of scoliosis, joint movement, cardiac valve disease and facial morphology [225].

Studies of ERT in animal models of MPS I, IIIB, VI and VII, showed that intravenously infused lysosomal enzymes are rapidly internalized by the liver, spleen and other peripheral tissues, however do not enter the brain parenchyma [226]. Sibling case studies of MPS I, II, and VI demonstrate much better outcome for younger siblings diagnosed at birth and started on ERT in the first six months of life [213,220,225,227].

The advantage of ERT includes improved walking ability, improved respiration and enhanced quality of life [212]. The downside for the clinical use of ERT are that the patients show no improvement in CNS pathology and there is a need for life-long intervention as high cost of the treatment [2]. The importance of NBS is proven as the earlier the patient is diagnosed with MPS, the earlier the patient can start with ERT, which is associated with lower mortality and morbidity [228], improved cognitive status [214,229] and a lower incidence of carpal tunnel syndrome(CTS) [230] (in children with MPS I) [212].

6.2 Hematopoietic Stem Cell Transplantation

In hematopoietic stem cell transplantation (HSCT), the enzyme is supplied endogenously through synthesis by the transplanted stem cells [212]. HSCT can treat the brain in some MPS disorders, especially if HSCT is given early to a patient as stem cells can engraft and differentiate in the CNS [212]. Whereas in infused ERT, it is too large a protein to cross the blood brain barrier (BBB) easily [212]. Stem cell sources for HSCT now include umbilical cord blood which is more readily available than bone

marrow, and has been proved safe and effective [231]. HSCT involves a toxic ablative conditioning regimen to eliminate the patient's own stem cell population, followed by immunosuppression and semi-isolation for up to 12 months [212]. HSCT for MPS shows improvements in physical activity and bone mineral density (BMD) of mice that were treated, and early interventions of HSCT showed further benefits [232–234].

HSCT has been used to treat patients with MPS I since 1981 [235,236] and is typically recommended for patients with Hurler syndrome under 2 years of age with normal cognition (DQ>70), as it can prolong survival rate, preserve neurocognition, and improve some somatic qualities [237]. HSCT is reserved for the most severe form of MPS I under 2 years of age with developmental quotient $\geq 70\%$ of normal [237] due to its significant morbidity and mortality [228,238,239].

HSCT is limited and the results are varied for other MPS disorders, as most case reports have reported only partial or none neurocognitive benefit [228,240–242]. HSCT can preserve cognition and prolong survival in very young children with the most severe form of MPS I [212]. The reason that HSCT is not as successful as it is in MPS I, as unlike MPS I, other MPS types are not typically diagnosed in infancy and so HSCT occurs usually past two years of age. This is when the CNS disease has been established and is usually irreversible [212]. For patients with MPS II, HSCT improves more activities of daily living (ADL) compared to ERT [243].

The two major challenges with HSCT is there is a difficulty in finding compatible stem cell donors and it is not entirely effective as it has a relatively high mortality rate due to graft versus host disease [244,245]. Both ERT and HSCT are not cures, but have been successful in altering the natural history of the disease [212].

6.3 Gene Therapy

Human gene therapy involves the insertion of normal DNA directly into cells to correct a disease-causing genetic defect [212]. Gene therapy can be performed in a variety of ways including a viral vector or ex-vivo approach including the removal of cells from the patient via blood or stem cells, genetically modifying them to produce the deficient enzyme and then re-introducing the genetically modified host cells by autologous transplant [246–248].

6.4 Substrate Reduction Therapy

An alternative approach to ERT is substrate reduction therapy (SRT). SRT is a treatment strategy that employs small molecule inhibitors to reduce the biosynthesis of storage metabolites, which accumulate in the absence of a specific lysosomal enzyme [92,249,250]. The progress in testing the efficacy of SRT for MPS in vivo has been hindered by a lack of specific inhibitors of GAG biosynthesis [251].

Chapter 7

MATERIALS AND METHODS

7.1 Subjects

For this experiment, we had a total of 5 MPS IVB patients, 34 MPS IVA patients, and 46 MPS II patients. The specific number of each type of MPS patients can be seen in the chart below.

7.2 Luminex

Immunoassays based on Luminex xMAP (multi-analyte profiling) technology allows the simultaneous detection and quantified of multiple secreted proteins such as cytokines, chemokines and growth factors [331]. Luminex is based on polystyrene or paramagnetic microspheres, also known as beads, that are dyed internally with red and infrared fluorophores of different intensities [332]. These bead sets are coated with a capture antibody that is qualified for one specific analyte. Each dyed bead is given a specific bead region, which allows the differentiation of one bead from another. These multiple analyte beads will be combined in a single well of a 96 well microplate. Luminex is very similar to ELISA testing, however it has a greater efficiency, speed, dynamic range and is less expensive per target result [331]. Eriko Yasuda, a previous graduate student in the Skeletal Dysplasia Lab performed all tests with Luminex and Tandem Mass Spectrometry for the biomarker assessment.

7.3 Tandem Mass Spectrometry

Tandem mass spectrometry (MS/MS) has evolved a powerful tool to detect small molecules, due to the direct measurement of each compounds based on the mass of the parent compound and a specific fragment [76]. Mass spectrometry methods are superior in accuracy, speed, sensitivity and specificity compared to other detection methods [82]. Tomatsu et al. developed a highly accurate, sensitive, and cost-effective liquid chromatography tandem mass spectrometry (LC-MS/MS) method to measure the four disaccharides of the GAGs; CS, DS, HS and KS [76].

The LC-MS/MS can assay DS, HS, and KS simultaneously in blood and/or urine samples [120,126,252,276,333–335]. Disaccharides were produced by specific enzyme digestion of each GAG, and are quantified by negative ion mode for multiple reaction monitoring [76]. These disaccharides of GAGs can be separated by liquid chromatography if these GAGs with the same molecular weights [76]. The LC-MS/MS method can show sensitivity and specificity for detecting all specific subtypes of MPS, and is able to monitor therapeutic efficacy in MPS patients and even animal models [123–126,276]. However, a limitation of the LC/MS/MS step is that it is time-consuming, which limits its utility of the mass spectrometry of NBS samples [76].

7.4 T-test

I was able to find the significance of the results for my experiment by performing a T-test. I used an alpha of 0.05 that is used as a cutoff for the significance of my experiment. If the p-values were less than 0.05, that meant I was able to reject

the null hypothesis that there is no different between the means. From this I was able to conclude that the significant difference does exist. If the p-value was larger than 0.05 then I could conclude that there is no significant difference between the MPS type and the normal control. The p-value can be seen in Figures 19-29.

N	EGF	IL-1b	IL-6	IL-10	MIP-1a	TNFa
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
MPS IVB	5	0	5	0	0	5
MPS IVA no treatment	26	17	20	2	16	23
MPS IVA ERT	9	0	9	3	0	9
MPS IVA TOTAL	35	17	29	5	16	32
MPS II no treatment	22	24	23	1	21	14
MPS II ERT	10	14	13	3	12	3
MPS II HSCT	5	5	5	2	5	3
MPS II TOTAL	37	43	41	6	38	20
Normal control	19	18	36	0	31	28

Table 4: Number of patients for EGF, IL-1b, IL-6, IL-10, MIP-1a and TNFa biomarkers.

N	MMP-1	MMP-2	MMP-9	Col II
	pg/ml	pg/ml	pg/ml	ng/mL
MPS IVB	0	5	5	5
MPS IVA no treatment	14	23	26	26
MPS IVA ERT	3	8	8	6
MPS IVA TOTAL	17	31	34	32
MPS II no treatment	18	24	24	23
MPS II ERT	10	14	12	15
MPS II HSCT	5	4	4	4
MPS II TOTAL	33	42	40	42
Normal control	37	30	28	39

Table 5: Number of patients for MMP-1, MMP-2, MMP-9 and Collagen II biomarkers.

N	DiHS0S	DiHSNS	DiHS6S	Mono-sulfated KS	Di-sulfated KS
	ng/ml	ng/ml	ng/ml	ug/ml	ug/ml
MPS IVB	5	5	5	5	5
MPS IVA no treatment	13	13	10	26	25
MPS IVA ERT	8	7	7	8	8
MPS IVA TOTAL	21	20	17	34	33
MPS II no treatment	21	23	20	23	25
MPS II ERT	14	14	11	13	15
MPS II HSCT	5	5	5	4	5
MPS II TOTAL	40	42	36	40	45
Normal control	35	41	39	43	44

Table 6: Number of patients for Heparan Sulfate and Keratan Sulfate biomarkers.

Chapter 8

RESULTS

EGF was significantly higher in MPS IVB, untreated MPS IVA, MPS IVA with ERT, MPS IVA all patients, untreated MPS II, MPS II with ERT and MPS with HSCT patients than that in normal controls.

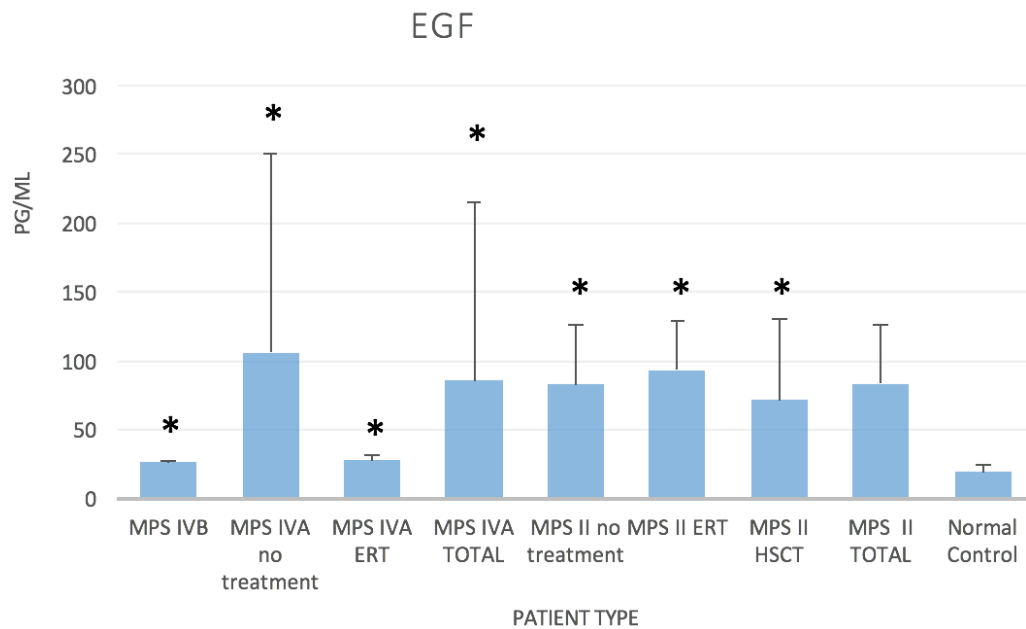


Figure 19: EGF biomarker levels in various types of MPS patients. * $p < 0.05$ vs normal control group.

IL-1 β on average was higher in patients with MPS compared to the normal control. In particular, untreated MPS IVA, MPS IVA all patients, untreated MPS II, MPS II with ERT and MPS II all patients are significantly higher compared to the normal control.

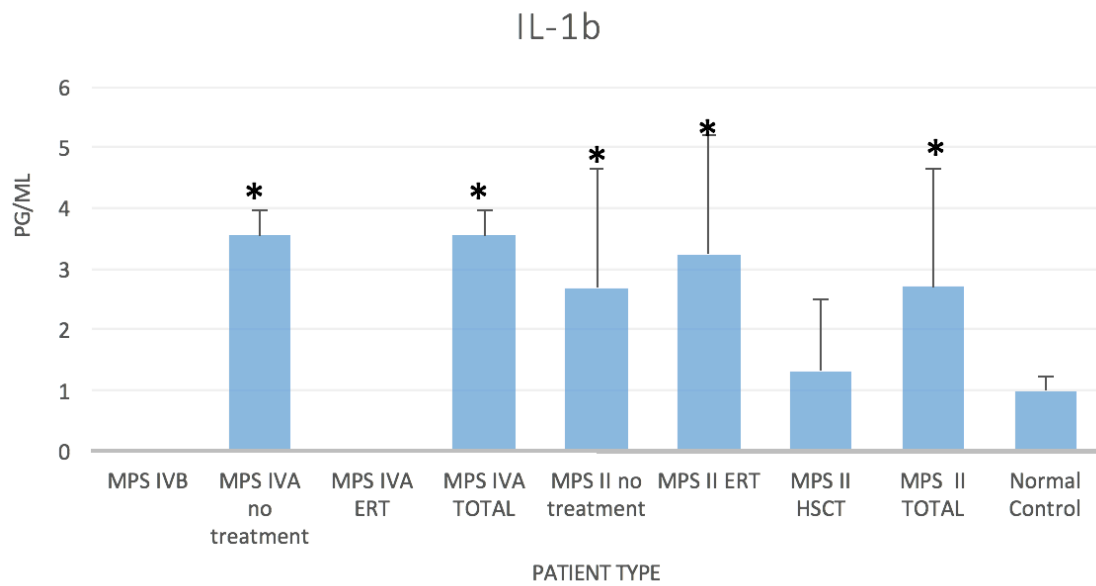


Figure 20: IL-1 β biomarker levels in various types of MPS patients.
*p<0.05 vs normal control group.

IL-6 was higher in patients with MPS on average compared to that in the normal controls. In particular, patients with MPS IVB, untreated MPS IVA, MPS IVA with ERT, MPS IVA all patients, untreated MPS II, MPS II with ERT and MPS II all patients are significantly higher compared to the normal control.

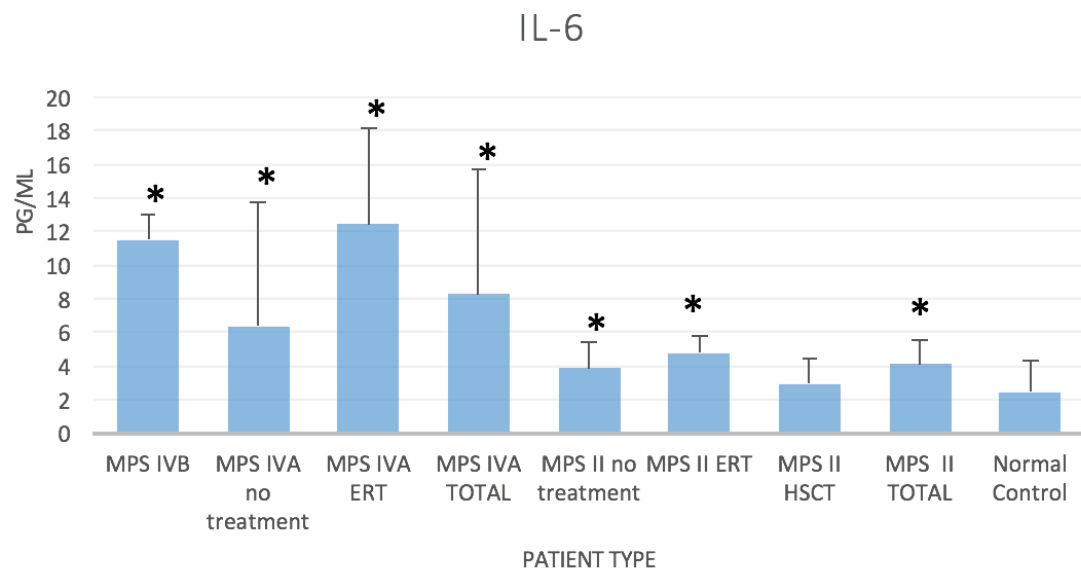


Figure 21: IL-6 biomarker levels in various types of MPS patients. * $p < 0.05$ vs normal control group.

MIP-1 α was significantly lower in patients with untreated MPS II, MPS II with ERT, and MPS II with HSCT and MPS II all patients compared to the normal control.

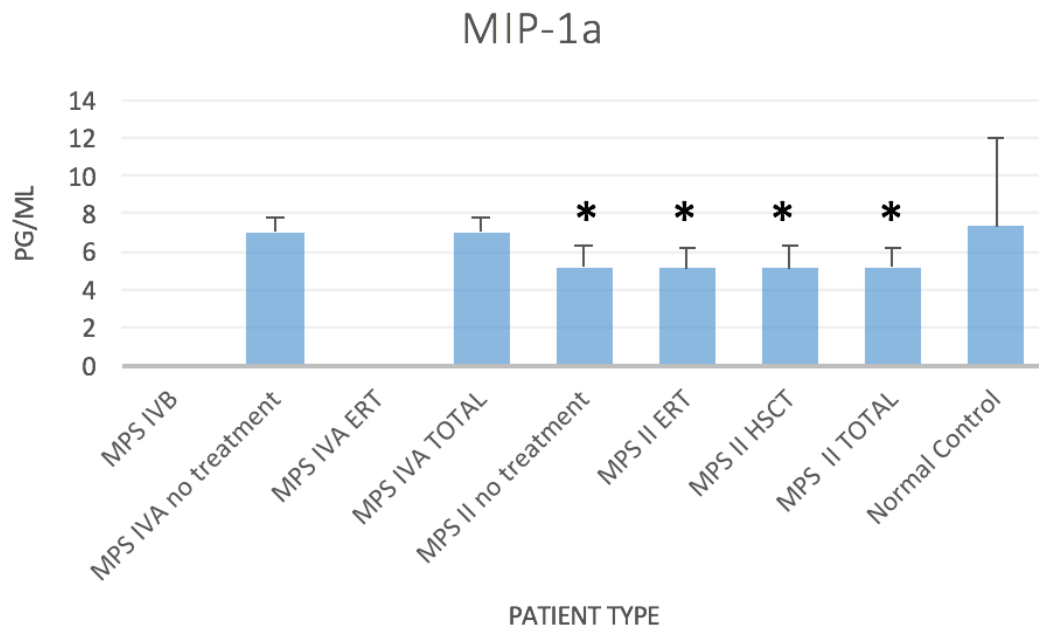


Figure 22: MIP-1 α biomarker levels in various types of MPS patients. *p<0.05 vs normal control group.

TNF- α was significantly higher in patients with MPS IVB, MPS IVA with ERT, and MPS IVA all patients compared to the normal control.

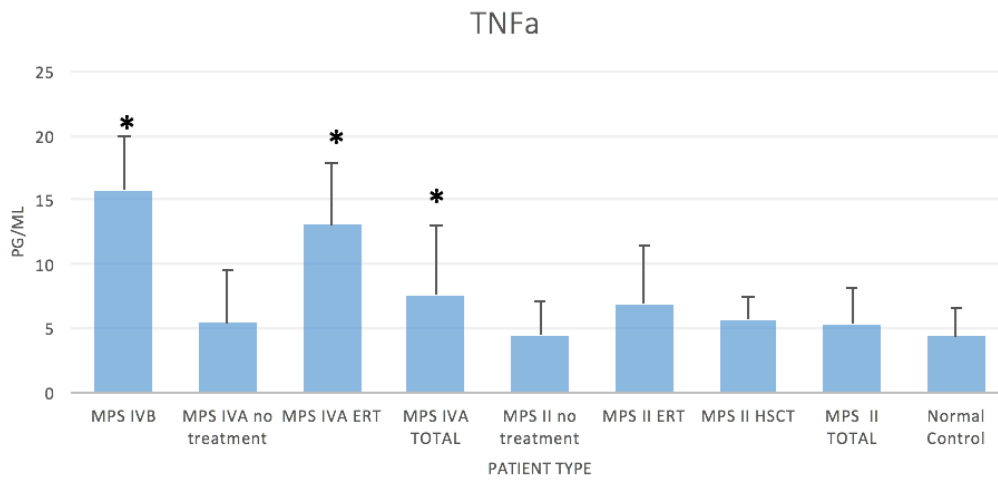


Figure 23: TNF α biomarker levels in various types of MPS patients. *p<0.05 vs normal control group.

MMP-1 was significantly higher in patients with untreated MPS IVA and MPS IVA all patients, and significantly lower in patients with untreated MPS II, MPS II with ERT, MPS II HSCT, and MPS II all patients compared to the normal controls.

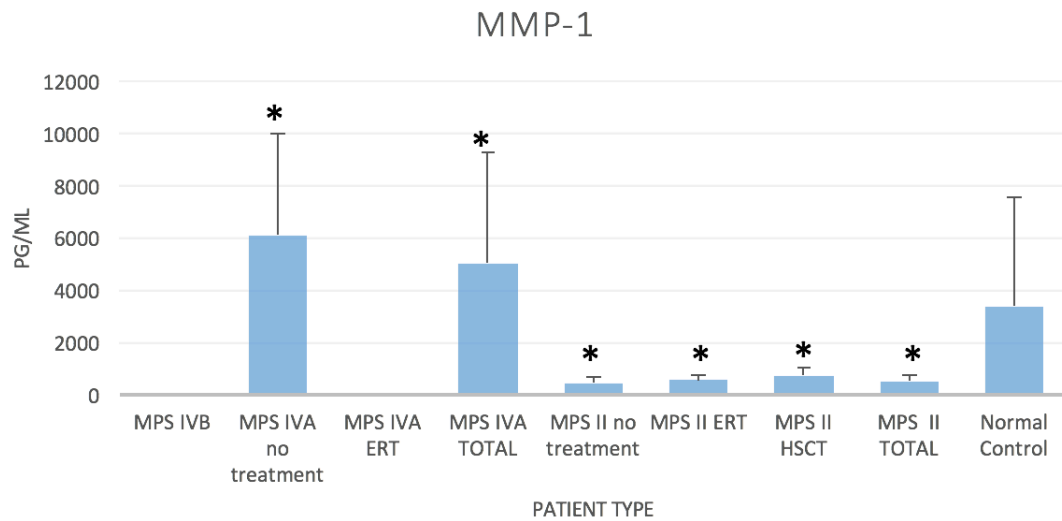


Figure 24: MMP-1 biomarker levels in various types of MPS patients. * $p < 0.05$ vs normal control group.

MMP-2 is significantly higher in untreated MPS IVA patients and MPS IVA all patients compared to the normal control.

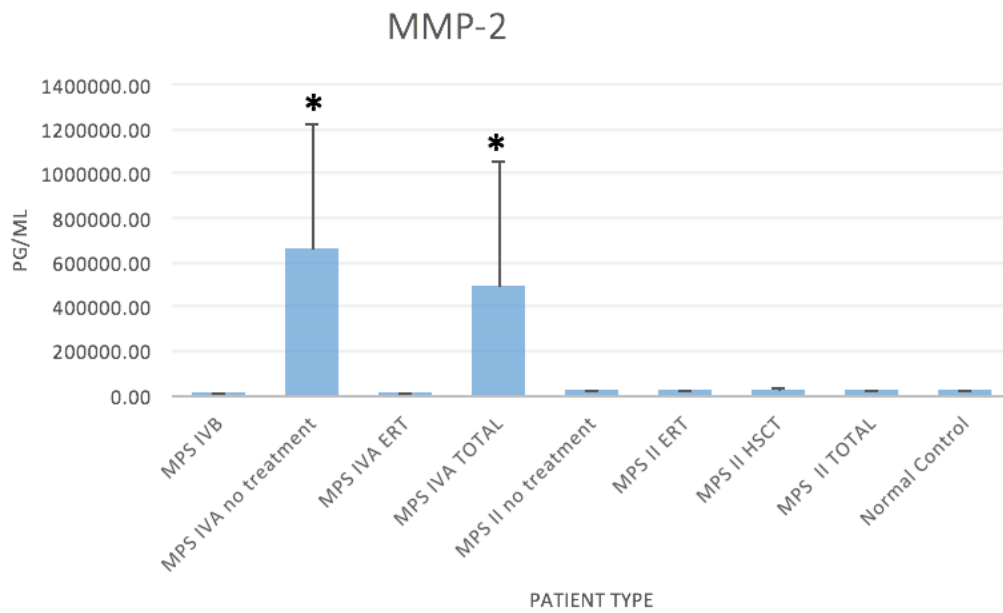


Figure 25: MMP-2 biomarker levels in various types of MPS patients. *p< 0.05 vs. normal control groups.

MMP-9 was significantly higher in patients, untreated MPS IVA and MPS IVA all patients and lower in MPS IVB patients and MPS IVA patients with ERT compared to the normal control.

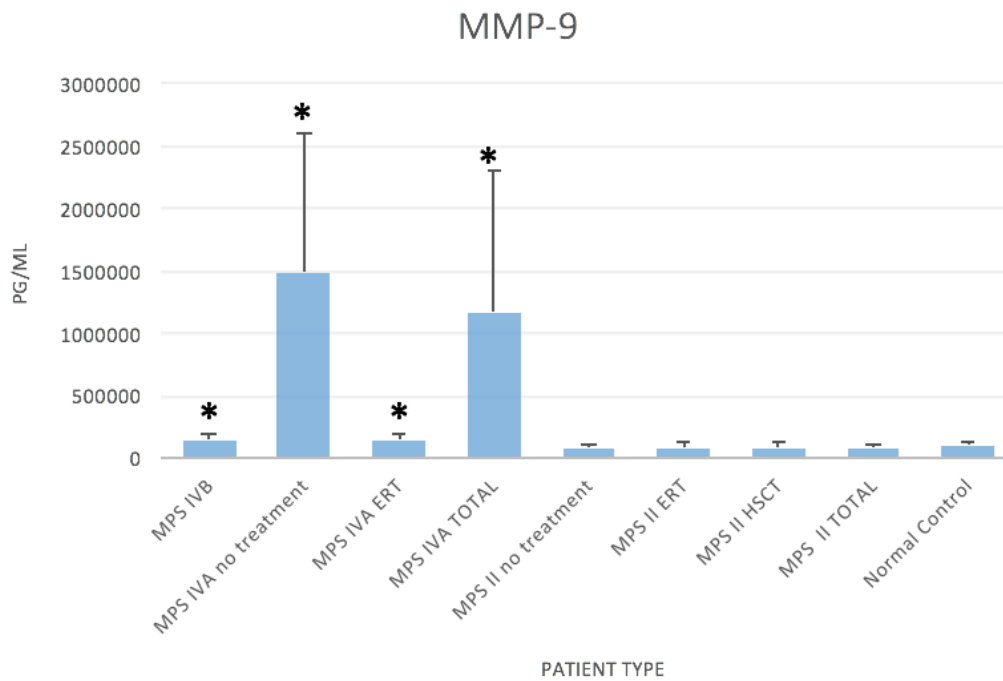


Figure 26: MMP-9 biomarker levels in various types of MPS patients. * $p < 0.05$ vs normal control group.

Collagen II was significantly decreased in untreated MPS IVA, MPS IVA all patients, untreated MPS II, MPS II with ERT and MPS II all patients compared to that of the normal control.

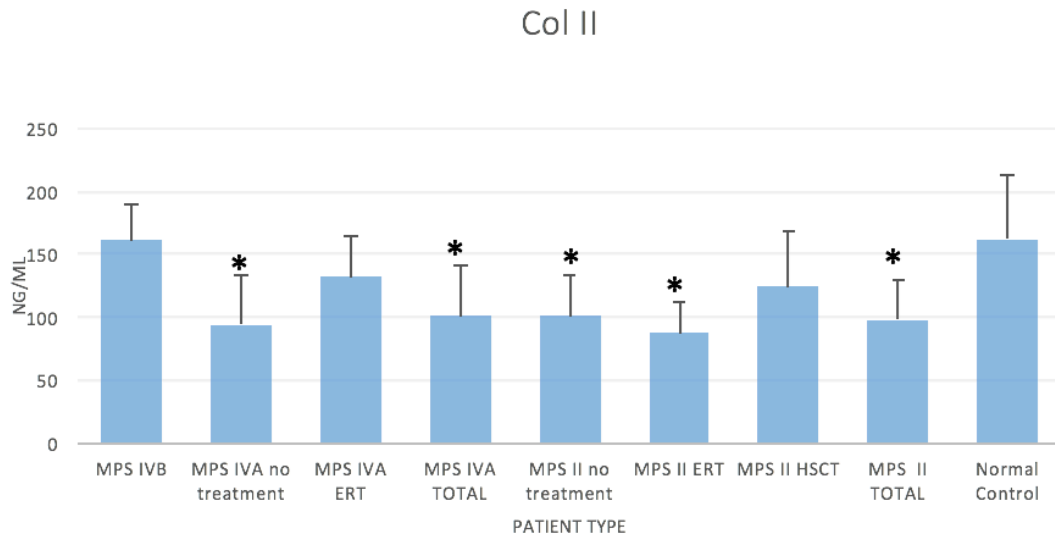


Figure 27: Collagen II biomarker levels in various types of MPS patients. * $p < 0.05$ vs. normal control groups.

Heparan Sulfate was significantly increased in patients with MPS II compared to that of the normal controls.

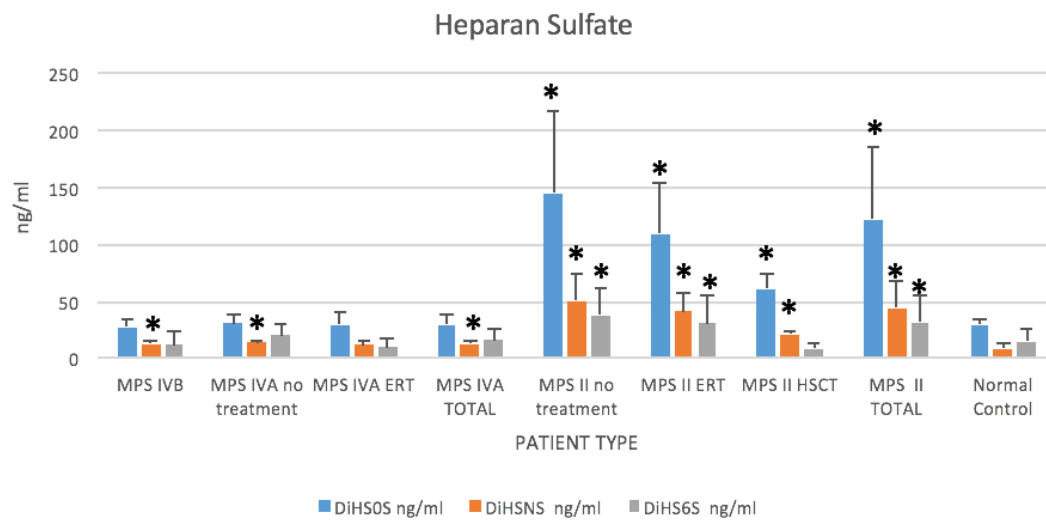


Figure 28: Heparan Sulfate biomarker levels in various types of MPS patients.
*p<0.05 vs. normal control groups.

Keratan Sulfate was higher in patients with MPS compared to that in the normal controls. On average, patients with MPS II, MPS IVA, and MPS IVB were significantly elevated compared to the normal controls.

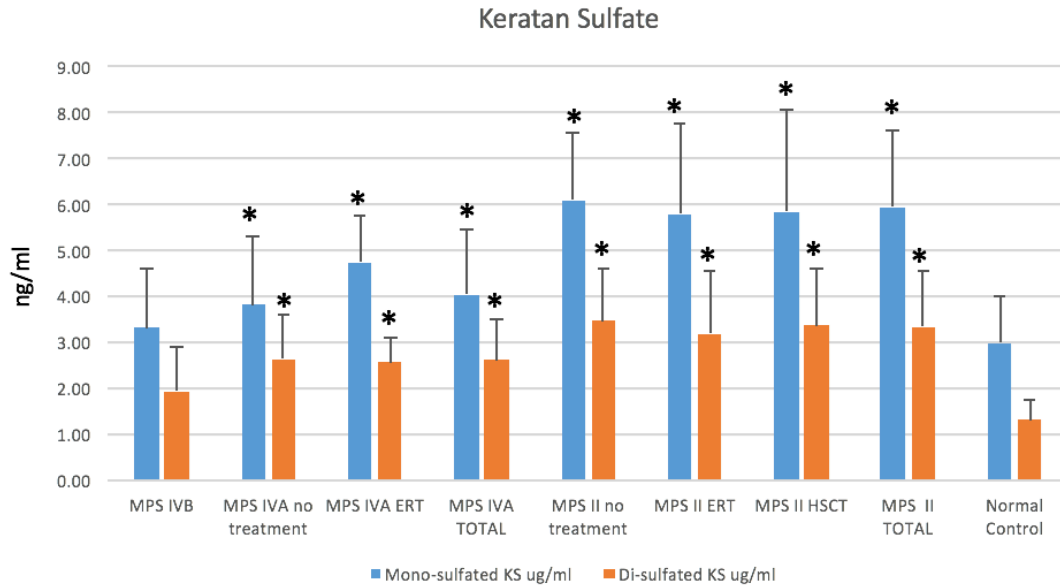


Figure 29: Keratan Sulfate biomarker levels in various types of MPS patients. *p<0.05 vs. normal control groups.

Chapter 9

DISCUSSION

In 2005, Simonaro et al. showed that GAG storage in MPS leads to cytokine elevation in MPS cells and animals. Jacques et al. also measured pro-inflammatory cytokines and showed elevation of IL-1 β and TNF- α in plasma from MPS II patients with ERT compared to controls [336]. IL-1 β positively correlated with TNF- α and nitric oxide (NO) in plasma [336]. Lysosomes are quite susceptible to oxidative stress, due to high iron content. Kharbanda et al. have demonstrated that levels of IL-1B, IL-6, IL-8, TNF- α , and MIP-1a from bronchoalveolar lavage (BAL) fluid samples were higher in patients with MPS on average.

Banecka et al. showed that an excessive EGF stimulates GAG synthesis in MPS fibroblasts, as it shows that under such conditions, accumulation of GAGs is more efficient in untreated patients [337]. Our findings positively correlate with those of previous findings, proving that EGF is higher in patients with MPS.

Although there are no previous findings of MIP-1 α in MPS IVB, IVA or MPS II patients, Natale et al. showed a tenfold increase of MIP-1 α in MPS IIIB mouse

serum compared with normal mice [314]. Our findings are new, yet important as it shows that MIP-1 α is a clear example for a biomarker for MPS patients.

Polgreen et al. indicated that TNF- α levels were significantly higher in children and adolescents with MPS, compared to the levels in healthy children. TNF- α was associated with pain and physical disability, despite treatment with ERT and/or HSCT [316]. Higher TNF- α levels correlated with more pain, decreased physical function, increased social limitations and overall decreased quality of life [316]. Our findings were matched that with previous studies as well, proving that TNF- α is an excellent biomarker for MPS patients.

Simonaro et al. reported that enhanced MMP immunostaining was seen in the cartilage and bone marrow of the MPS animals, and that enhanced MMP expression was primarily detected in the superficial and intermediate zones, and bone marrow osteoclasts and osteoblasts [295]. Enhanced expression of tissue inhibitor of MMP-1, MMP-2, and MMP-9 was observed in the MPS animals, associated with abnormal expression of TIMP-1. Expression of MMPs was significantly enhanced in articular chondrocytes [295]. Batzios et al. have indicated that MMP-2 expression was significantly increased in serum, whereas a significant decrease in serum MMP-9 was observed in MPS III patients [328]. For MPS II patients, there was a 2.6-fold increase of MMP-2 level in serum and a 4-fold decrease in MMP-9 level [328].

The study by de Francheschi et al. showed low expression of collagen type II at protein and molecular levels in MPS IVA patients [338]. Our findings also correlate with MPS II, IVA and IVB patients that collagen II is also a clear biomarker for MPS patients.

Chapter 10

CONCLUSION

Overall, pro-inflammatory cytokines, chemokines, and GAGs are potential biomarkers for diagnosis, prognosis, and therapeutic efficacy in patients with MPS. Having relatively small number of patients with MPS in the current study is a limitation in our findings; however, it sets a pathway for further testing to be performed. Our findings prove that GAGs are very important biomarkers for diagnosis, prognosis, and monitoring therapies of MPS patients. Our findings also show the importance of testing for biomarker levels in patients with MPS. Our results correlate to what previous research findings are. Overall, MPS II, MPS IVA, and MPS IVB patients are significantly elevated in biomarkers compared to the normal control patients.

It is important that biomarkers be tested for patients with MPS, as little research has been done for MPS, and testing for biomarkers are important for bettering

the quality of life of patients with MPS. By finding the biomarkers of MPS, we are better able to help patients with MPS long term.

For future studies, data from more patients including other types of MPS should be accumulated. We suggest having similar age groups for MPS patients and for the normal control patients to be tested, instead of having a large variety of age group in both the MPS patients and the normal control patients, as was performed in my thesis. Moving forward, patients should be separated based on gender, having a different result table for girls vs. boys affected with MPS.

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Appendix A

PUBLISHED MANUSCRIPTS

Peracha, H., Sawamoto K., Averill L., Kecskemethy H., Theroux M., Pizarro C., Mackenzie W., Orii T., Tomatsu S., “Diagnosis and Prognosis of Mucopolysaccharidosis IVA”. 2018

Peracha, H., Khan S., Wiesbaur, A., Keuhni, C., Rohrbach, M., Mason, R., Giugliani, R., Suzuki, Y., Orii, K., Tomatsu, S. “Epidemiology of Mucopolysaccharidosis”. 2017