Molecular Genetics and Metabolism 109 (2013) 301–311

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Pathogenesis of Morquio A syndrome: An autopsied case reveals systemic storage disorder

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ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form 8 April 2013
Accepted 9 April 2013
Available online 16 April 2013

Keywords:
Pathogenesis
Mucopolysaccharidosis IVA
Keratan sulfate
Chondroitin-6-sulfate
Cervical fusion

ABSTRACT

Mucopolysaccharidosis IVA (MPS IVA; Morquio A syndrome) is a lysosomal storage disorder caused by deficiency of N-acetylgalactosamine-6-sulfate sulfatase, which results in systemic accumulation of glycosaminoglycans (GAGs), keratan sulfate and chondroitin-6-sulfate. Accumulation of these GAGs causes characteristic features as disproportionate dwarfism associated with skeletal deformities, genu valgum, pigeon chest, joint laxity, and kyphoscoliosis. However, the pathological mechanism of systemic skeletal dysplasia and involvement of other tissues remain unanswered in the paucity of availability of an autopsied case and successive systemic analyses of multiple tissues.

We report here a 20-year-old male autopsied case with MPS IVA, who developed characteristic skeletal features by the age of 1.5 years and died of acute respiratory distress syndrome five days later after occipito-C1–C2 cervical fusion. We pathohistologically analyzed postmortem tissues including trachea, lung, thyroid, humerus, aorta, heart, liver, spleen, kidney, testes, bone marrow, and lumbar vertebrae.

The postmortem tissues relevant with clinical findings demonstrated 1) systemic storage materials in multiple tissues beyond cartilage, 2) severely vacuolated and ballooned chondrocytes in trachea, humerus, vertebrae, and thyroid cartilage with disorganized extracellular matrix and poor ossification, 3) appearance of foam cells and macrophages in lung, aorta, heart valves, heart muscle, trachea, visceral organs, and bone marrow, and 4) storage of chondrotin-6-sulfate in aorta.

This is the first autopsied case with MPS IVA whose multiple tissues have been analyzed pathohistologically and these pathological findings should provide a new insight into pathogenesis of MPS IVA.

1. Introduction

Mucopolysaccharidosis IVA (MPS IVA; Morquio A syndrome) is a lysosomal storage disease (LSD), caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). Deficiency of this enzyme causes accumulation of specific glycosaminoglycans (GAGs), keratan sulfate (KS) and chondroitin-6-sulfate (C6S), mainly in cartilage and its extracellular matrix (ECM), and leads to incomplete endochondral ossification [1–7]. MPS IVA is characterized clinically by disproportionate dwarfism, dysostosis multiplex with universal platyspondyly, anterior beaking of lumbar spine, pectus carinatum, flaring of the rib cage, epiphyseal dysplasia of joints, knock-knee, laxity of joints, corneal

Abbreviations: ARDS, acute respiratory distress syndrome; BMI, body mass index; CDC, Centers for Disease Control and Prevention; Cr, creatinine; C6S, chondroitin-6-sulfate; ECM, extracellular matrix; EM, electron microscopy; EVG, elastic tissue fibers-van Gieson; GALNS, N-acetylgalactosamine-6-sulfate sulfatase; GAG, glycosaminoglycan; H&E, hematoxylin and eosin; KS, keratan sulfate; LSD, lysosomal storage disease; LM, light microscopy; MPS IVA, mucopolysaccharidosis IVA; PAS, periodic acid-Schiff; TB, toluidine blue.

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http://dx.doi.org/10.1016/j.ymgme.2013.04.009

opacities, dental abnormalities, cardiopulmonary complications, normal intelligence, and elevation of blood and urine KS [1–8]. Many patients become wheelchair-bound in their second decade and undergo multiple orthopedic surgeries to alleviate serious medical complications [5–7]. Mortality and morbidity are commonly due to the respiratory failure by obstructive and restrictive lung and spinal cord injury by spinal cord compression and instability of C1–C2 joint [5–7,9]. Articular and growth cartilage is the main place of bone pathology in MPS IVA where the endochondral ossification is distorted [5–7,10,11]. In spite of the devastating cartilage disease, there has been only a few data reporting histological and molecular evaluations of bone and cartilage pathology in MPS IVA patients [10,11].

One of the reasons is due to lack of an autopsied case with MPS IVA. Although two autopsied cases were reported in 1970s, none of these cases were diagnosed enzymatically and only brain pathology was described in details [12,13]. The fetal case with MPS IVA was also reported in 1992, showing that resting chondrocytes and placental villus have multiple vacuoles by electron microscopy (EM) and that the accumulation of storage materials starts in the fetus [14]. In biopsied cartilage of patients with MPS IVA, a low expression of collagen type II and a high expression of collagen type I were found out, speculating that these findings could lead to laxity of joints [10]. The ECM of cartilage in MPS IVA patients is affected, providing an impact on the phenotypic properties of chondrocytes and resulting in the formation of cartilage more prone to degeneration with an explanation for the occurrence of osteoarthritis at an early stage [11]. Zustin recently proposed that the early pathogenesis in MPS IVA could be because of the inadequate regression of cartilage canals and impaired resorption and restitution of pannus tissue [15].

However, to date there was no autopsied case with MPS IVA describing pathology of the multiple tissues systemically, therefore, we could not elucidate the pathogenic mechanism of the skeletal dysplasia and involvement of other tissues.

It is critical to investigate tissues affected with MPS IVA, leading to the complete understanding of pathogenic mechanism of the disease and to future development of the unique therapies for MPS IVA.

The purpose of this study is to report morphologic and pathologic observations of tissues from an autopsied case.

2. Material and methods

2.1. Tissues analyzed

We autopsied a 20-year-old male case with MPS IVA at Gifu University. We analyzed postmortem tissues including trachea, lung, thyroid, humerus, aorta, heart, liver, spleen, kidney, testes, bone marrow, and lumbar vertebrae by light microscopy (LM) and EM. Informed consent for the autopsy was obtained and tissue preparations for pathological analyses were conducted at Gifu University.

2.2. LM

Samples of bone and soft tissues were fixed in 10% neutral buffered formalin for 24–36 h. Bone samples were decalcified in Regular Cal Immuno™ (BBC Biochemicals, Mount Vernon, WA), washed in running water and checked for end-point decalcification. All tissues were auto-processed through graded ethanol, cleared in Safe-Clear™ (Thermo-Fisher, Kalamazoo, MI), and embedded in paraffin. Sections were cut at 5 μm, floated onto poly-lysine coated slides, and heat-immobilized for 1 h at 60 °C. Slides were cooled to room temperature prior to staining. All sections were deparaffinized, hydrated to distilled water and stained with hematoxylin and eosin (H&E), colloidal iron/van Gieson and Alcian blue/periodic acid-Schiff (PAS), and elastic tissue fibers-van Gieson (EVG) stains. H&E stains were performed on a Sakura DRS-601 automated stainer using a standard H&E protocol. Colloidal iron stains were conducted manually using a modified Mowery's technique [16]. The slides were stained for Alcian blue/PAS as follows: tissue sections were placed in a 2.5 pH Alcian blue solution for 30 min. Excess stain was blotted from the slides and placed in a 1% periodic acid solution for 10 min. Sections were then rinsed in running tap water for 5 min, placed in Schiff's reagent for 10 min, and subsequently washed for 10 min in lukewarm water. EVG staining was performed as follows: the slides were stained in Verhoeff's hematoxylin for 30 min and differentiated in 2% ferric chloride solution, followed by counterstaining by van Gieson's for 5 min.

All slides were dehydrated in graded ethanol, cleared in Histo-Clear™ (National Diagnostics, Atlanta, GA) and cover-slipped in permount.

H&E staining portrays the morphology of the cell. Colloidal iron stains acidic mucopolysaccharides in blue, while van Gieson stains collagen in pink or deep red. In Alcian blue staining, acidic mucopolysaccharides become blue. In PAS staining, sugars and neutral mucopolysaccharides are stained in red. EVG staining was used for identifying elastic fibers and collagen in aorta. The elastic fibers were stained blue-black and collagen was stained pink-red.

The C6S antibody immunostaining were performed for aorta and foam cells.

2.3. EM

The formula of fixative for EM is as follows; 0.1 M cacodylic acid formaldehyde (final Ca.1.5%) and glutaraldehyde (final 1%) (pH 7.2–7.4 adjusted by HCl). Following aldehyde fixation, tissue was washed in 0.1 M sodium cacodylate buffer pH 7.2 containing 5% sucrose, and was post-fixed in 1% osmium tetroxide in cacodylate buffer containing 1% sucrose. Excess osmium was washed out with cacodylate buffer and distilled water, and the tissue was dehydrated with graded ethanol and propylene oxide. Following infiltration with EmBed resin, blocks were polymerized at 80 °C, and thin sections were cut with Leica EM UC7 ultramicrotome, collected on copper grids and post-stained with uranyl acetate and lead citrate. Electron micrographs were obtained using a JEOL 1200EX EM. For evaluation of lysosomal storage, toluidine blue (TB)-stained 0.5-μm-thick sections of tissue were assessed by LM and EM.

2.4. KS level

We obtained plasma and urine samples at the age of 12 years and analyzed KS level in blood and urine by ELISA method [17].

3. Results

3.1. Case report (Fig. 1)

This Japanese patient was born without any complication, and his birth length and weight were 55 cm and 3832 g above the average of Japanese birth height and weight. He was noticed of short stature, prominent forehead, pigeon chest, and abnormal gait by the age of 1.5 years. He was diagnosed with scoliosis and osteomalacia at the beginning, diagnosed as chondrodysplasia. Prominent forehead, marked short stature, neck, knock-knee, chest deformities, hyperlaxity of joints, and corneal clouding developed with age. He was diagnosed enzymatically as Morquio A at 5 years and 4 months at Gifu University. He received cervical decompression/fusion at 6.5 years old; however, cervical fusion was not successful. The patient underwent bone marrow transplantation at 12 years old whose donor was his elder brother; however, he could not obtain engraftment. At the age of 13 years, aortic valve insufficiency was pointed out, and the patient was completely wheel-chair bound with shortness of breath. Since 19 years of age, gait disturbance got worse with muscle weakness and loss of stamina, leading to the complete wheel-chair bound situation, and severe cervical spinal cord compression was observed by CT and MRI (Fig. 2). His genotype was c.758+1G>C (p.R253fsX1)/c.871G>A (p.A291T).