Molecular Genetics and Metabolism Reports 14 (2018) 59–67

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

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

Case Report

Natural history of Morquio A patient with tracheal obstruction from birth to death

Caitlin Doherty[a,b], Lauren W. Averill[a], Mary Theroux[a], William G. Mackenzie[a], Christian Pizarro[a], Robert W. Mason[a], Shunji Tomatsu[a,c,d,*]

[a] Nemours/Alfred I. duPont Hospital for Children, Wilmington, DE, USA
[b] University of Delaware, Newark, DE, USA
[c] Department of Pediatrics, Gifu University, Gifu, Japan
[d] Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA, USA

ARTICLE INFO

Keywords:
Natural history
Morquio A syndrome
Tracheal obstruction
Autopsy
Keratan sulfate

ABSTRACT

Morquio A syndrome (mucopolysaccharidosis IVA, MPS IVA) is a lysosomal storage disease caused by a deficiency of *N*-acetylgalactosamine-6-sulfate sulfatase, resulting in systemic accumulation of the partially degraded glycosaminoglycans (GAGs), keratan sulfate and chondroitin-6-sulfate. The accumulation of these GAGs leads to distinguishing features as skeletal dysplasia with disproportionate dwarfism, short neck, kyphoscoliosis, pectus carinatum, tracheal obstruction, coxa valga, genu valgum, and joint laxity. In the absence of autopsied cases and systemic analysis of multiple tissues, the pathological mechanism of the characteristic skeletal dysplasia associated with the disease largely remains a question. Here we report an autopsied case of a 23-year-old male with MPS IVA, who developed characteristic skeletal abnormalities by 4 months of age and died of severe tracheal obstruction and hypoventilation originating from respiratory muscle weakness from neurological cord deficit due to cord myelopathy at the age of 23. We analyzed postmortem tissues pathohistologically, including the thyroid, lung, lung bronchus, trachea, heart, aorta, liver, spleen, kidney, testes, humerus, knee cartilage, and knee ligament.

Examination of the tissues demonstrated systemic storage materials in multiple tissues, as well as severely ballooned and vacuolated chondrocytes in the trachea, humerus, knee cartilage, and lung bronchus.

This autopsied case with MPS IVA addresses the importance of tracheal obstruction for morbidity and mortality of the disease, and the pathological findings contribute to a further understanding of the pathogenesis of MPS IVA and the development of novel therapies.

1. Introduction

Morquio A syndrome (mucopolysaccharidosis IVA, MPS IVA) is a lysosomal storage disease (LSD) caused by a deficiency of *N*-acetylgalactosamine-6-sulfate sulfatase (GALNS), which is required for the catabolism of glycosaminoglycans (GAGs): keratan sulfate (KS) and chondroitin-6-sulfate (C6S) [1–7]. As a result, partially degraded GAGs accumulate in bone, ligaments, and cartilage, as well as the extracellular matrix (ECM) of these tissues, impeding endochondral ossification and chondrogenesis [2,3,8]. In looking at the bone pathology of MPS IVA, endochondral ossification is primarily distorted at articular and growth cartilage [2,8,9]. MPS IVA ranges from mild to severe, with systemic bone dysplasia often increasing the severity of the disease [8,9]. MPS IVA is characterized by extensive clinical manifestations including skeletal dysplasia with prominent forehead, dental abnormalities, short neck, short trunk dwarfism, cervical spinal cord compression and atlantoaxial instability, tracheal obstruction, kyphoscoliosis, pectus carinatum, pulmonary complications, laxity of joints, coxa valga, genu valgum, and elevated blood and urine KS [1–3,8,9]. In addition, radiographic findings display failure of ossification, cervical spinal stenosis with dysplasia of odontoid process, platyspondyly of vertebral bodies, flaring of the rib cage, anterior beaking of the lumbar bodies, tilted ulna, epiphyseal dysplasia of joints, flaring iliaca, and flattened femoral head. While most patients appear normal at birth, major skeletal abnormalities often develop within a few years of age [1]. Individuals with MPS IVA, particularly with the severe form, often do not survive past their twenties, as the most attributed causes of mortality and morbidity are spinal cord compression, instability of the C1-C2 joint, and airway compromise including tracheal obstruction [1–3,8,9].

* Corresponding author at: Skeletal Dysplasia Center, Nemours Biomedical Research, Nemours/Alfred I. DuPont Hospital for Children, 1600 Rockland Rd., Wilmington, DE 19803, USA.
E-mail address: stomatsu@nemours.org (S. Tomatsu).

https://doi.org/10.1016/j.ymgmr.2017.11.005
Received 30 September 2017; Received in revised form 27 November 2017; Accepted 27 November 2017

Therefore, most patients require several surgeries to alleviate severe orthopedic complications, such as cervical spinal decompression and fusion, limb osteotomy, hemiepiphysiodesis, and hip reconstruction/replacement; nonetheless, they often become wheelchair-dependent by their second decade [1–3,8,9].

Tracheostomy has often been required for tracheal obstruction, and due to a difficult airway, patients remain high risk for anesthesia, making intubation and extubation challenging or impossible [3,10]. Difficult airways in MPS IVA patients arise from the imbalance of growth between the trachea and vessels vs. cervicothoracic spine and ribs, short neck, large tongue and mandible, hypertrophic adenoid and tonsils, and the angle of manubrium as a result of GAG deposits in chondrocytes and their ECM [2,17,18]. A study examining tracheal obstruction in 28 MPS IVA patients with the severe phenotype revealed that 67.9% of patients showed tracheal narrowing, and that tracheal narrowing worsened with age (all 8 patients over the age of 15 had > 50% tracheal narrowing [3,7]. Eight out of the 28 patients presented with severe (> 75%) tracheal narrowing. This tracheal narrowing was found to be most attributed to compression by the tortuous brachiocephalic artery and was evident as early as at the age of 2 years old. Performing a tracheostomy can prove difficult in MPS IVA patients due to a tortuous and redundant trachea, short neck, and inability to hyperextend the neck. Airway obstruction due to vascular compression and a narrowed thoracic inlet requires an alternative method. Pizarro et al. describe a novel surgical procedure for tracheal obstruction without the need for a tracheostomy, which should improve difficult airway and narrowed trachea [11].

As MPS IVA remains a systemic skeletal disorder without a cure, there have been few histological and molecular evaluations of cartilage and bone pathology in MPS IVA patients, particularly due to the lack of autopsied cases [2]. While two autopsied cases were presented in the 1970s, only brain pathology was reported, and neither case was diagnosed enzymatically [2,12,13]. A fetal case with MPS IVA described in 1992 showed that electron microscopy (EM) depicted that placental villi and resting chondrocytes have multiple vacuoles, with the accumulation of storage materials starting in the fetus. Biopsied cartilage of MPS IVA patients indicates a high expression of collagen type I and a low expression of collagen type II, with the speculation that this type of collagen expression seen could lead to laxity of joints [2,14]. Cartilage ECM of MPS IVA patients is also affected, impacting the phenotypic properties of chondrocytes, and causing the development of cartilage that is more prone to degradation [2,15]. This provides an explanation for the early occurrence of osteoarthritis. It was also recently proposed that the inadequate regression of cartilage canals and impaired resorption and restitution of pannus tissue could be the cause of early pathogenesis in MPS IVA [2,16]. In 2013, Yasuda et al. reported pathologic and morphologic findings of tissues from an autopsied case with MPS IVA 8 years post-failed HSCT, which was the first incidence in which the pathology of multiple tissues was described systemically.

Currently, we are unable to fully explain the pathogenic mechanism of the characteristic skeletal dysplasia associated with MPS IVA as well as the widespread involvement of other tissues [2]. In order for the development of therapies that could benefit patients with MPS IVA, it is imperative that we gain comprehensive knowledge regarding the pathogenesis of the disease through the examination of affected tissues.

In this article, we have described the pathologic observations of tissues from an autopsied case with severe tracheal obstruction and respiratory muscle weakness arising from neurological cord deficit due to cord myelopathy to further understand the pathogenesis of MPS IVA.

2. Materials and methods

2.1. Tissues analyzed

A 23-year-old male with MPS IVA was autopsied at the University of Tennessee. Informed consent for the autopsy was obtained, and tissue preparations for the pathological analyses were conducted at Alfred I. duPont Hospital for Children. We examined postmortem tissues including the heart, aorta, liver, trachea, thyroid, lung, lung bronchus, spleen, kidney, testes, humerus, knee ligament, and knee cartilage by light microscopy (LM).

2.2. Light microscopy (LM)

Bone and soft tissue samples were fixed for 24–36 h in 10% neutral buffered formalin. Bone samples were then decalcified in Regular Cal Immuno™ (BBC Biochemicals, Mount Vernon, WA), washed in running water, and examined for end-point decalcification. Tissues were auto-processed through graded ethanol, cleared in Safe-Clear™ (Thermo-Fisher, Kalamazoo, MI), and then embedded in paraffin. 5 μm sections were cut, floated onto poly-lysine coated slides, and heat-immobilized at 60° C for 1 h. Prior to staining, slides were cooled to room temperature. Sections were deparaffinized, hydrated with distilled water, and stained with colloidal iron/ van Gieson, hematoxylin and eosin (H&E), Alcian blue/periodic acid-Schiff (PAS), toluidine blue (TB), and Safranin O (Saf O). Using a standard H&E protocol, H&E stains were conducted on a Sakura DRS-601 automated stainer. Colloidal iron stains were performed manually through the use of a modified Mowery's technique [2]. The Alcian Blue/PAS staining method is as follows: tissues were placed in an Alcian blue solution of pH 2.5 for 30 min. Excess stain was removed from the sides and then placed in a periodic acid solution for 10 min. The sections were rinsed in running tap water for 5 min, placed in Schiff's reagent for 10 min, and washed for another 10 min in lukewarm water. For Safranin O, tissues were stained with Safranin O and fast green. All of the slides were dehydrated in graded ethanol, cleared in Histo-Clear™ (National Diagnostics, Atlanta, GA) and cover-slipped in permount. Colloidal iron stains acidic mucopolysaccharides a dark blue colour, while van Gieson stains collagen pink. H&E staining demonstrates the morphology of the cell, with hematoxylin staining nuclei in blue and eosin staining the cytoplasm in pink. Alcian blue staining turns acidic mucopolysaccharides blue, while PAS staining turns sugars and neutral mucopolysaccharides red. Safranin O stains nuclei black, the cytoplasm gray/green, and cartilage and acidic proteoglycans red.

For evaluation of lysosomal storage by light microscopy, toluidine blue-stained 0.5-μm thick sections were examined. The formula of fixative for toluidine blue (TB) is as follows; 0.1 M cacodylic acid formaldehyde (final ca.1.5%) and glutaraldehyde (final 1%) (pH 7.2–7.4 adjusted with HCl). Following infiltration with EmBed resin, blocks were polymerized at 80 °C, and thin sections were cut with Leica EM UC7 ultramicrotome.

3. Results

3.1. Case report (Figs. 1–2)

This Caucasian patient was born without complications at 38-weeks gestation, with a birth weight and length of 2.72 kg and 43.2 cm, respectively. While he began sitting at 5 to 6 months of age, crawling at 7 to 8 months and walking at 13 months, thoracolumbar kyphosis and mild dextroconvex scoliosis were detected at 4 months of age. By 2 years old, the patient had exhibited further anomalies including lordosis and short stature, which led to an enzymatically confirmed diagnosis of MPS IVA at the age of 3 years and 2 months old at the University of Tennessee, Medical Center. At the time of his diagnosis, the patient had shown worsening kyphosis and scoliosis as well as vision and hearing difficulties, eventually requiring glasses and bilateral hearing aids. Prominent forehead, hearing loss, corneal clouding, dental problems, large tongue, short neck, chest deformities, laxity of joints, and decreased stamina developed with age. The patient was found to have severe cervical cord compression at the C-1 level at 4 years old, resulting in posterior decompression and occipito-C1-C2