Urinary karatan sulfate (uKS) in Morquio syndrome type A patients measured via LC-MS/MS method: Improved KS detection as compared to dye-based methods and report of age-specific uKS reference ranges

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Morquio syndrome type A (mucopolysaccharidosis type IVA, MPS IVA) is a lysosomal disorder caused by insufficient N-acetylgalactosamine-6-sulfatase (GALNS) activity. The lysosomal enzyme GALNS catabolizes the glycosaminoglycans (GAG) keratan sulfate (KS) and chondroitin 6-sulfate (C6S), and deficient GALNS activity causes accumulation of KS and C6S. While demonstration of deficient GALNS enzyme activity is the gold standard for diagnosis of Morquio syndrome type A, detection of elevated urinary GAG (GAG) levels is often used to screen for Morquio syndrome type A. However, standard dye-based analyses of total GAG levels (the quantitative GAG test) have a high false negative rate in the identification of patients with Morquio syndrome type A, in some cases leading to misdiagnoses. False positives are also possible. To address the use of the liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based urine KS (uKS) test as an improved method to measure KS in comparison with dye-based methods, two international data steering committees were organized by BioMarin Pharmaceutical Inc. (Nashville, USA and Bahia, Brazil) in 2014. Case data on age-specific reference ranges for uKS levels in unaffected individuals and patients with Morquio syndrome type A were accumulated and the performance of the dye-based GAG test and LC-MS/MS uKS tests in identifying patients with Morquio syndrome type A were compared. Multiple independent international labs assessed uKS levels by the dye-based GAG test and the LC-MS/MS uKS test in unaffected individuals and patients with Morquio syndrome type A. We confirm previous findings that the levels of uKS decrease with age in unaffected individuals. In particular, creatinine-normalized uKS levels are highest in children less than 1 year of age and then decrease rapidly. While results from the dye-based test and the LC-MS/MS uKS test were linearly correlated, we highlight multiple cases where the superior sensitivity of the LC-MS/MS uKS test resulted in identification of patients with Morquio syndrome type A while the dye-based test did not. Although uKS has, to date, not been shown to be an efficacy biomarker for enzyme replacement therapy, it is an excellent screening biomarker for Morquio syndrome type A and potentially for other disorders. In contrast to dye-based methods, the LC-MS/MS-based uKS test provides a highly sensitive, specific, and quantitative measure of elevated KS. Optimally, LC-MS/MS-based GAG measurements will enable more accurate urine screening for MPS disorders.

Mucopolysaccharidosis (MPS) VII is associated with severe musculoskeletal manifestations, which are particularly prevalent in the spine. Vertebral dysplasia and accelerated intervertebral disc degeneration lead to kyphoscoliosis and spinal cord compression, directly impacting patient mortality and quality of life. A defining feature of spine disease in MPS VII is the presence of cartilaginous lesions in the vertebral bodies, indicative of failed cartilage-to-bone conversion during postnatal development [1,2]; however, the underlying molecular mechanisms are poorly understood. During normal vertebral bone formation, cartilaginous rudiments form a template where resident chondrocytes undergo distinct stages of differentiation (resting, proliferative, hypertrophic, terminal) regulated by a highly orchestrated pattern of growth factor signaling, culminating in ossification by osteoblasts. GAGs perform critical roles in regulating the activity of these growth factors. We hypothesize that abnormal GAG accumulation in MPS VII disrupts chondrocyte differentiation by interfering with growth factor signaling, thus preventing normal cartilage-to-bone conversion. Our objectives were to: 1) identify the earliest developmental age where altered bone formation is evident in MPS VII, and 2) establish the stage at which epiphyseal chondrocyte differentiation is impaired, using the naturally-occurring MPS VII canine model. We collected thoracic vertebrae from 9 day (n = 2) and 14 day old (n = 5) litter-matched heterozygote and affected dogs for quantitative PCR and micro-computed tomography (microCT) analyses. This age range was selected based on previous radiographic, longitudinal studies of vertebral bone formation in MPS VII dogs [2]. Comparison of mRNA expression of chondrocyte differentiation (SOX9, RUNX2, COL10A1) and osteoblast (ALPL, BGLAP) markers, and microCT visualization of vertebral bodies of heterozygote and MPS VII affected dogs showed striking differences in bone formation (*, figure) at 14 days whereas at 9 days, no significant differences besides a trend towards lower RUNX2 expression were detected (Figure). Interestingly, SOX9 expression was downregulated at 14 days for both heterozygote and affected dogs suggesting that both chondrocyte populations receive regulatory signals for proliferation but that MPS VII chondrocytes fail to progress into hypertrophy. Furthermore, bone volume fraction and bone mineral density quantification of the vertebral bone primary ossification centers showed no differences between heterozygote and affected dogs suggesting that at this time point, the most affected developmental pathways involve activation of secondary ossification centers. Since the epiphyses at these early ages are composed of cartilage and therefore high in GAG content, aberrant GAG accumulation in MPS VII is likely playing a role in failed ossification. Indian Hedgehog signaling plays a crucial role in regulating chondrocyte differentiation and is regulated in a highly GAG-dependent manner [3,4,5]. Our preliminary data showed differences in Hedgehog pathway mRNA expression levels between heterozygote and affected animals at both 9 and 14 days implicating this pathway in disease etiology (figure). The results of this study lay the foundation for future mechanistic investigations into bone disease in all MPS subtypes.

References: