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# Characterization of the MPS I-H knock-in mouse reveals increased femoral biomechanical integrity with compromised material strength and altered bone geometry

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ABSTRACT

Mucopolysaccharidosis type I (MPS I), is an autosomal recessive lysosomal storage disorder caused by a deficiency in the α-L-iduronidase enzyme, resulting in decreased enzymatic activity and accumulation of glycosaminoglycans. The disorder phenotypically manifests with increased urine glycosaminoglycan excretion, facial dysmorphology, neuropathology, cardiac manifestations, and bone deformities. While the development of new treatment strategies have shown promise in attenuating many symptoms associated with the disorder, the bone phenotype remains unresponsive. The aim of this study was to investigate and further characterize the skeletal manifestations of the *Idua*-W392X knock-in mouse model, which carries a nonsense mutation corresponding to the IDUA-W402X mutation found in Hurler syndrome (MPS I-H) patients. μCT analysis of the microarchitecture demonstrated increased cortical thickness, trabecular number, and trabecular connectivity along with decreased trabecular separation in the tibiae of female homozygous *Idua*-W392X knock-in ($IDUA^{-/-}$) mice, and increased cortical thickness in male $IDUA^{-/-}$ tibiae. Cortical density, as determined by μCT, and bone mineral density distribution, as determined by quantitative backscattered microscopy, were equivalent in $IDUA^{-/-}$ and wildtype (Wt) bone. However, tibial porosity was increased in $IDUA^{-/-}$ cortical bone. Raman spectroscopy results indicated that tibiae from female $IDUA^{-/-}$ had decreased phosphate to matrix ratios and increased carbonate to phosphate ratios compared to Wt female tibiae, whereas these ratios remained equivalent in male $IDUA^{-/-}$ and Wt tibiae. Femora demonstrated altered geometry and upon torsional loading to failure analysis, female $IDUA^{-/-}$ mouse femora exhibited increased torsional ultimate strength, with a decrease in material strength relative to Wt littermates. Taken together, these findings suggest that the $IDUA^{-/-}$ mutation results in increased bone torsional strength by altering the overall bone geometry and the microarchitecture which may be a compensatory response to increased porosity, reduced bone tensile strength and altered physiochemical composition.



## 1. Introduction

Mucopolysaccharidosis type I (MPS I; MIM# 252800) is an autosomal recessive lysosomal storage disorder caused by a deficiency in the lysosomal enzyme α-L-iduronidase (EC 3.2.1.76), which catalyzes the degradation of the glycosaminoglycans (GAGs), dermatan sulfate and heparan sulfate [1]. The clinical severity of MPS I is dependent upon α-L-iduronidase activity, which can vary widely and has been categorized into three distinct phenotypic subtypes: MPS I-Hurler (MPS I-H; MIM# 607014), MPS I-Scheie (MPS I-S; MIM# 607016), and MPS I-

*Abbreviations:* MPS I, mucopolysaccharidosis type I; IDUA, α-L-iduronidase; GAGs, glycosaminoglycans; μCT, microcomputed tomography; BMDD, bone mineral density distribution; FWHM, full width at half maximum; BV/TV, bone volume/total volume; SMI, structure model index; $T_{max}$, torsional ultimate strength; Su, tensile strength; U, energy to failure; Ks, stiffness; G, shear modulus of elasticity; BMD, bone mineral density.

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Hurler/Scheie (MPS I-H/S; MIM# 607015), with the clinical severity ranging from MPS I-H being the most severe subtype to MPS I-S being the least severe. The incidence of MPS I-H is estimated to occur in 1:100,000 births [2]. The accumulation of GAGs results in progressive cellular damage and visual impairment, hearing loss, cardiac manifestations, organomegaly, developmental delay with subsequent progressive cognitive decline initiated by age 2 in severe MPS I-H patients [3]. Furthermore, MPS I-H patients present with severe skeletal abnormalities through poorly understood mechanisms [4]. The skeletal abnormalities are collectively known as dysostosis multiplex, and consist of stiffness and contracture of joints, enlarged skull, genu valgum, thoracolumbar kyphosis, hip dysplasia, abnormally shaped vertebrae and ribs, hypoplastic epiphyses and short stature [5–8]. As a result of the skeletal manifestations, MPS I-H patients typically undergo multiple high-risk surgical interventions to delay the progression of the skeletal disease and improve quality of life [9–11]. Novel therapeutic treatments such as bone marrow or umbilical cord blood transplantation and weekly enzyme replacement are currently being used to sustain overall enzyme activity and have improved and extended the quality of life of Hurler patients. However, these treatments are unable to fully prevent development of the skeletal manifestations in Hurler patients [9,12]. Therefore, despite these therapeutic gains, MPS-I patients continue to endure the consequences of disabling, painful bone disease that often require rigorous surgical intervention.

In contrast to the two previously reported *Idua* knock-out mouse models, *Idua* −/− and MPS I [13,14], the *Idua*-W392X knock-in mouse model carries a nonsense mutation corresponding to the *IDUA*-W402X mutation, commonly found in Hurler syndrome patients [15]. Wang et al. showed that the phenotype of *Idua*-W392X mouse model parallels that of human MPS I-H disease, which includes GAG accumulation due to loss of α-L-iduronidase activity, cardiac manifestations and bone abnormalities such as broadening of the face, thickening of the zygomatic arch and atypical femur length and width [15]. Even though the existing animal models have been useful in evaluating various therapeutic approaches such as enzyme replacement therapy [16], bone marrow transplantation [17,18], and gene therapy [19,20], much of the molecular mechanisms leading to the pathology remain to be elucidated. Although it has been shown that the *Idua*-W392X presents with typical skeletal findings of MPS I-H [15,21], the whole bone biomechanical and material properties have not been evaluated. The paucity in understanding the pathogenic mechanisms responsible for the musculoskeletal manifestations likely contributes to the absence of improvement in the bone phenotype with the current therapies.

The goal of this study was to evaluate the microarchitecture, physiochemical composition and biomechanical integrity of the *Idua-W392X* knock-in mouse model, in order to better define the underlying biomechanical properties of the skeletal abnormalities and to begin to elucidate the physiochemical mechanisms. Femora and tibiae from wildtype (Wt), heterozygous *Idua*-W392X ($IDUA^{+/-}$) and homozygous *Idua*-W392X ($IDUA^{-/-}$) 16 week old mice were evaluated on the basis of whole bone biomechanical integrity and material properties. This study highlights the microarchitectural, physiochemical, and biomechanical basis of the MPS I-H skeletal phenotype and can be used to drive forward the design and improvement of current and future therapies to improve the bone quality of MPS I-H patients.

## 2. Methods

### 2.1. Animals and tissue harvest

The *Idua-W392X* mice were a generous gift from Dr. Kim Keeling, University of Alabama at Birmingham, Alabama [15]. *Idua-W392X* mice were previously backcrossed into a C57BL/6J congenic background [17]. $IDUA^{+/-}$ breeding pairs and their offspring were housed in an AAALAC accredited facility at the University of Missouri-Columbia and had ad libitum access to water and food (Purina 5008 Formulab Diet; Purina Mills Inc., St. Louis, MO). This study was performed under an approved University of Missouri Animal Care and Use Protocol. The breeders and offspring were genotyped as previously described [17] and weighed weekly starting at 5 weeks of age. The mice were raised to 16 weeks of age (peak bone mineral density [22]), sacrificed, and femora and tibiae were excised, the soft tissue removed, and the bones wrapped in sterile 1× PBS soaked gauze and stored at −20 °C until analyzed.

### 2.2. Tibial microarchitecture analysis

The macro- and microarchitecture of the left tibiae was determined by microcomputed tomography (μCT) with the vivaCT 40 (Scanco Medical AG, Bassersdorf, Switzerland) as previously described [23] using 55 kVp X-ray tube potential, 145 μA current, 10 μm voxel resolution, and 200 ms integration time to assess cortical bone and trabecular bone properties. By hydroxyapatite calibration, the voxel values were converted to a mineral-equivalent value, milligrams per cubic centimeter ($mg/cm^3$). Three dimensional images of the tibiae were reconstructed along the long axis with series of 10 μm-thick slices, using a global threshold of 253 (μCT gray value). The tibial cortical density and thickness were evaluated in the transverse plane at the mid-shaft starting 1 mm proximal to the fibula–tibia junction. The proximal metaphysic trabecular bone was analyzed 1 mm below the growth plate, and the following determined: the bone volume fraction (BV/TV), trabecular bone density, thickness, number, separation, connectivity density, which describes abundance of trabecular connections, and the structure model index (SMI), which describes shape of individual trabecular bone ranging from 0 (perfect plate) to 3 (cylindrical rods) [24,25].

### 2.3. Tibial bone mineral density distribution and porosity

Quantitative backscattered scanning electron microscopy of left tibias was used to characterize the bone mineral density distribution (BMDD) and porosity. Tibiae were scanned in a digital electron microscope containing a four-quadrant semiconductor backscattered detector (Evo, Ziess, Germany). Imaging was performed at a 20 kV accelerating voltage, saturated filament current, 0.5 nA probe current measured with a Faraday cup and a working distance of 12 mm. Magnification settings, store resolution and scan speed were kept consistent between different imaging sessions to result in a pixel size of 760 nm. Several high magnification images were taken from the cross section of each sample and the images were combined using an image processing software (Image composite editor, Microsoft Research) to form the whole bone cross section. To calibrate the backscattered signal, pure carbon and aluminum standards (Micro-Analysis Consultants, UK) were imaged in each scan and at the same condition of imaging the bone. Calibration standards were imaged in several intervals while scanning the bone. After imaging, the graylevel numbers of standards were averaged between each two subsequent images of the phantom to account for the signal variations. In a post-processing analysis and to keep the scale of the graylevel histograms consistent between images, graylevel numbers of the bone and phantom were expanded linearly such that carbon and aluminum were 25 and 225, respectively. A histogram containing the incidence of graylevel numbers was calculated for each sample and the mode and the full width at half maximum (FWHM) of the histograms were determined. The bone porosity was determined by excluding regions with cracks and microcracks from the bone cross-section and then determining the fraction of the bone area with lacunae and blood vessels relative to the total bone area (Fig. 3A–C).

### 2.4. Bone mineral and matrix composition

Following μCT analyses the left tibia was sliced across the diaphyseal midshaft and the cortical bone cross-sections were evaluated by Raman