National Institutes of Health (NIH)

Adeno-Associated Virus 8 human N-acetylgalactosamine-6-sulfate-sulfatase (AAV8-hGALNS)

Rare Pediatric Disease Designation Request

June 25, 2024

TABLE OF CONTENTS

Section **Page**

LIST OF ABBREVIATIONS

AAV: Adeno-associated virus
AAV8: Adeno-associated virus serotype 8
AAV8-hGALNS: Adeno-associated virus serotype 8 human N-acetylgalactosamine-6-sulfate sulfatase
ADL: Activities of Daily Living
AEs: Adverse events
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
CBER: Center for Biologics Evaluation and Research
cDNA: Complementary deoxyribonucleic acid
CS: Chondroitin sulfate
CT: Computed tomography
C6S: Chrondroitin-6-sulfate
DRG: Dorsal root ganglion
DS: Dermatan sulfate
ERT: Enzyme replacement therapy
EU: European Union
FDA: U.S. Food and Drug Administration
GAGs: Glycosaminoglycans
GALNS: N-acetylgalactosamine-6-sulfate sulfatase
GLP: Good laboratory practice
HS: Heparan sulfate
HSCT: Hematopoietic stem cell transplantation
IND: Investigational new drug
INTERACT: Initial Targeted Engagement for Regulatory Advice on CBER Products
IV: Intravenously
LBTP2: Liver-bone tandem promoter
LMTP6: Liver-muscle tandem promoter
LSD: Lysosomal storage disorder
KO: Knock out
KS: Keratan Sulfate
MPS: Mucopolysaccharidoses
MPS IVA: Mucopolysaccharidosis IVA, or Morquio Syndrome
MRI: Magnetic resonance imaging
M6P: Mannose-6-phosphate
NCATS: National Center for Advancing Translational Sciences
NHS: Natural history study
NIH: National Institutes of Health
ODD: Orphan Drug Designation

OTAT: Office of Tissues and Advanced Therapies
PFT: Pulmonary function test
POC: Proof of concept
QoL: Quality of Life
ROA: Route of administration
SCC: Spinal Cord Compression
TBG: Thyroxin-binding globulin

1 RARE PEDIATRIC DISEASE DESIGNATION REQUEST STATEMENT

Pursuant to 21 CFR 316.20, National Institutes of Health (NIH) requests designation of adeno-associated virus serotype 8 human N-acetylgalactosamine-6-sulfate sulfatase (AAV8-hGALNS) as an orphan drug product for treatment of patients with mucopolysaccharidosis IVA (MPS IVA) resulting from a deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS).

MPS IVA (Morquio A Syndrome or Morquio Syndrome type A) is a rare, debilitating, monogenetic lysosomal storage disease caused by defects in the gene coding for the enzyme, GALNS (Gene MIM number, 612222; EC 3.1.6.4). Reduced GALNS activity results in impaired catabolism of two glycosaminoglycans (GAGs), Chrondroitin-6-sulfate (C6S) and Keratan Sulfate (KS). Accumulation of C6S and KS manifests as systemic progressive skeletal dysplasia (dysostosis multiplex). More than 200 different mutations have been identified in the GALNS gene, which likely explains the phenotypic heterogeneity of the disorder. The onset of disease symptoms commonly occurs before age 1 year in patients with the severe form of MPS IVA or as late as the second decade of life in patients with attenuated MPS IVA [1].

Regardless of the rate of disease progression, all patients have serious and debilitating morbidities, including bone deformities, neurological complications from spinal cord compression and cervical myelopathy, and airway obstruction from tracheal narrowing [1]. Reconstructive trachea surgery, which involves the removal of several redundant trachea segments, has been performed to address this life-threatening condition. Although this surgery has provided MPS IVA patients with dramatic respiratory improvements, it is a high risk, especially when patients present with spinal cord compression.

Approximately 75% of cases present the classic phenotype exhibiting initial symptoms before age 1 year, with diagnosis before age 5 years [1]. These patients, if left untreated, have a decreased life expectancy and die of respiratory failure (63% of MPS IVA patients), cardiac failure (11%), and myocardial infarction (4%) [2]. Life expectancy in patients with rapidly progressing phenotypes ranges from 20 to 30 years. Rarely, patients with slowly progressing forms of the disorder survive beyond age 60. Reliable incidence figures are unavailable in the US; estimates have varied between 1 in 200,000 live births and 1 in 300,000 live births [3]. Based on the annual US birth rate of ~3.7 million/per year in 2021, approximately 12.3-18.5 children are born in the US with MPS IVA each year.

Previous Orphan Drug Designations (ODDs) for MPS IVA have been granted for lysosomal enzyme N-acetylgalactosamine-6-sulfate sulfatase in 2008 and elosulfase alfa in 2009, but there is still an unmet need for this patient population regarding effective treatment options, given the rarity of the disease and the small patient population size. The accumulated data indicate that ERT may reduce fatigue and improve patients' quality of life (QoL), but they have not shown any impact on cartilage, bone pathology, or bone growth.

Based on our good-faith assessment from the enrollment in our clinic on MPS IVA (over 100 patients) and data from an NIH-conducted natural history study, we estimate ~400 MPS IVA patients live in the US. Thus, the MPS IVA population potentially amenable to therapy with AAV8-hGALNS gene therapy is of very low prevalence, with an estimated number of patients ranging from 290 to 460 patients in the US, below the 200,000-prevalence cut-off to qualify for an Orphan designation. Therefore, we request that MPS IVA be designated as a Rare Pediatric Disease.

2 ADMINISTRATIVE INFORMATION

2.1 Sponsor

National Center for Advancing Translational Sciences, National Institutes of Health
9609 Medical Center Dr. Suite 1-E-162 Rockville, MD 20850

2.2 Contacts

Regulatory Correspondent

Sponsor Contact

Principal Investigator

2.3 Drug Name

2.3.1 Chemical Name - Drug Substance

Adeno-Associated Virus 8 human N-acetylgalactosamine-6-6sulfate sulfatase (AAV8-hGALNS)

2.3.2 Generic/Trade Name - Drug Product

AAV8-hGALNS is an Adeno-Associated Virus 8 (AAV8) vector expressing a functional human codon-optimized cDNA encoding the N-acetylgalactosamine-6-sulfate sulfatase (GALNS) under the control of liver-specific promoter.

2.4 Manufacturer's Name and Address

2.4.1 Drug Substance Manufacturer

Forge Biologics
3900 Gantz Road, Grove City, OH 43123
CDMO and FEIN To Be Confirmed

2.4.2 Drug Product Manufacturer

Astellas Gene Therapies
6074 Enterprise Park Dr, Sanford, NC 27330
CDMO and FEIN To Be Confirmed

3 DESCRIPTION OF RARE DISEASE OR CONDITION, PROPOSED INDICATION, AND NEED FOR THERAPY

3.1 Description of Rare Disease or Condition

Molecular etiology

Mucopolysaccharidosis IVA (MPS IVA or Morquio A Syndrome) is a rare, debilitating, monogenetic lysosomal storage disease caused by defects in the gene coding for the enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS; Gene MIM number, 612222; EC 3.1.6.4). The GALNS gene is located on chromosome 16q24 [4]. The isolation and characterization of the full-length cDNA encoding the human GALNS protein and genomic sequence [5] have facilitated investigations into the molecular genetic heterogeneity of MPS IVA. More than 200 different mutations have been identified in the GALNS gene, which likely explains the phenotypic heterogeneity of the disorder [6-8].

GALNS is a monomeric glycoprotein containing 522 amino acids and 2 potential N-linked glycosylation sites. GALNS functions to cleave O-linked sulfate moieties from both keratan sulfate (KS) and chondroitin-6-sulfate (C6S); therefore, due to the deficiency of GALNS, these molecules progressively accumulate in MPS IVA. Accumulation of C6S and KS manifests as systemic progressive skeletal dysplasia (dysostosis multiplex). GAGs accumulate in lysosomes, intracellular matrix, and extracellular matrix. MPS is categorized into 8 groups, with 12 different deficient lysosomal enzymes [9]. KS is closely involved in cellular motility, embryo implantation, wound healing, corneal transparency, and nerve regeneration [10]. C6S is also involved in embryo development, maintaining mechanical skin strength and cell proliferation.

Enzymes that escape the typical molecular routing system are secreted by the cell via the constitutive secretory pathway and are often recaptured by cell surface M6P receptors that return the GALNS to the lysosome by the endocytic pathway [11]. It is this aspect of lysosomal enzyme trafficking that makes AAV gene therapy a feasible therapeutic strategy for patients with MPS

IVA. The AAV gene therapy results in the production of the enzymes in transduced cells, secreting the enzymes in circulation and delivering them to the target sites. AAV gene therapy producing GALNS in patients with MPS IVA offers a safe and efficacious approach to this disease's treatment.

Onset and progression

The onset of disease symptoms commonly occurs before age 1 in patients with the severe form of MPS IVA or as late as the second decade of life in patients with attenuated MPS IVA [1,8]. Approximately 75% of cases present the classic phenotype exhibiting initial symptoms before age 1, with diagnosis before age 5 [1]. These patients, if left untreated, have a decreased life expectancy and die of respiratory failure (63% of MPS IVA patients), cardiac failure (11%), and myocardial infarction (4%) [2]. If untreated, life expectancy in patients with rapidly progressing phenotypes ranges from 20 to 30 years. Rarely, patients with slowly progressing forms of the disorder survive beyond age 60 [1].

Regardless of the rate of disease progression, all patients have severe and debilitating morbidities that can begin to manifest as early as age 5. The extracellular matrix of cartilage in MPS IVA patients is affected and impacts the phenotypic properties of chondrocytes, resulting in the formation of cartilage more prone to degeneration at an early age. Bone deformity is the most common initial manifestation of skeletal dysplasia [1,12-13]. Additional compromised systems include the auditory, cardiovascular, and respiratory systems [14]. The central nervous system is not believed to have significant manifestations of GAG accumulation, and normal intelligence appears preserved [15]. However, patients have a high risk of developing neurological complications caused by a combination of odontoid hypoplasia, incomplete ossification of the anterior and posterior rings of the atlas, and deposition of GAGs in the anterior extradural space. This results in atlantoaxial subluxation and spinal cord compression (SCC), with cervical myelopathy and consequential quadriparesis [14,16].

Height as important measurement

Most MPS IVA patients appear healthy during the neonatal period and manifest clinical symptoms reflecting a spectrum of progression from a severe “classical” phenotype to a mild “attenuated” phenotype [17-19]. While the clinical phenotype for categorizing severity has not been established, height based on age may be the most objective measurement. The mean height for males and females with MPS IVA at age 18 was 120.4 and 114.8 cm; these values correspond to −8.0 SD and −7.7 SD of the height for normal healthy males and females, respectively, according to growth charts from the Centers for Disease Control and Prevention [8,12,20]. Children with severe MPS IVA show a reduced growth rate beginning at approximately 18 months, and growth will stop at approximately age 10-11; however, some patients with attenuated MPS IVA may continue growing into adolescence and exceed 140 cm in height [12,20]. MPS IVA patients above the 90th percentile on the Morquio-specific growth chart are more likely to be defined as attenuated, less

than the 75th centile are defined as severe, and between the 75th and 90th centiles are defined as intermediate [12,20].

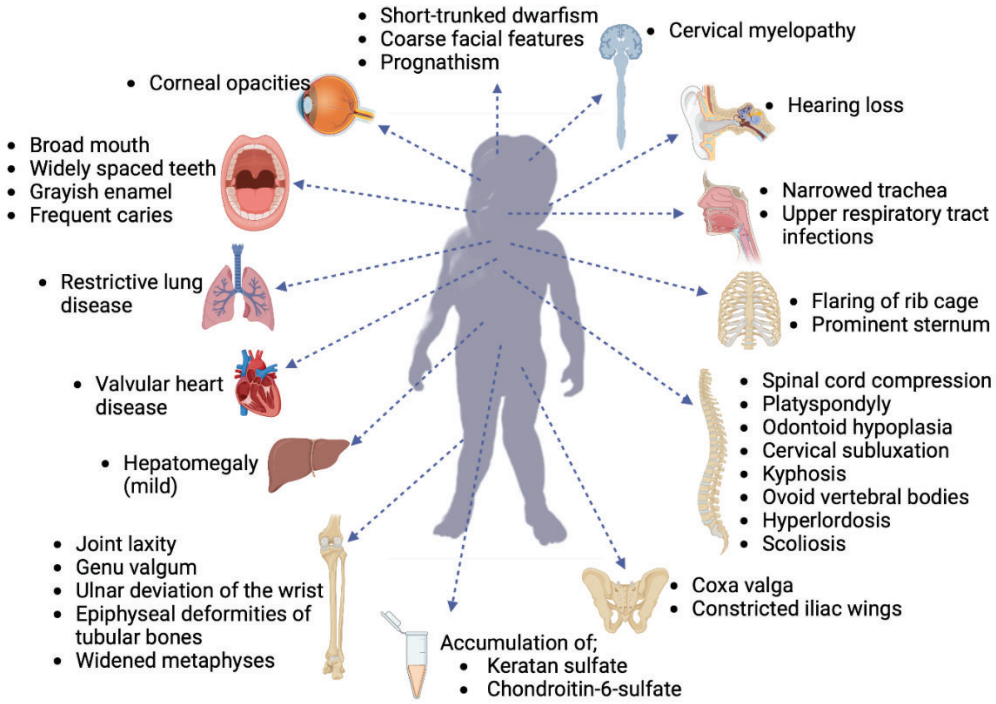

Figure 1. Clinical and Laboratory Manifestations of Mucopolysaccharidosis IVA

Other symptoms and clinical manifestation (Fig. 1)

The clinical phenotype of MPS IVA is due to the chronic and progressive accumulation of glycosaminoglycans (GAGs) in nearly all cell types, tissues, and organs of the body. MPS IVA is characterized by GAG accumulation in the respiratory tract, heart, liver, spleen, bones, joints, oropharynx, head, neck, and ligaments [1,8]. MPS IVA causes skeletal dysplasia through excessive storage of keratan sulfate (KS). Patients with MPS IVA appear healthy at birth. MPS IVA-specific radiographic changes prior to clinical symptoms and signs have been observed.

The initial symptoms and signs in most MPS IVA patients have been noticed by the age of three years. MPS IVA patients are usually evaluated for unusual skeletal features during the second or third year of life. These include striking short trunk dwarfism, odontoid hypoplasia, pectus carinatum, kyphosis, gibbus, scoliosis, genu valgus, coxa valga, flaring of the lower ribs, hypermobile joints, and abnormal gait with a tendency to fall. Patients with MPS IVA can usually be clinically distinguished from patients with other MPSs because they preserve intelligence and have additional skeletal manifestations derived from a unique spondyloepiphyseal dysplasia and ligamentous laxity. Odontoid hypoplasia, a bony vertebral projection at the C2 vertebrae, is the most critical skeletal feature to be recognized in any patient with MPS IVA. This in combination

with ligamentous laxity and extradural mucopolysaccharide deposition, results in atlantoaxial subluxation, cervical myelopathy, or even death.

Other common symptoms include diminished endurance with muscle atrophy and weakness, gait abnormalities due to hip dysplasia with genu valgum, thoracolumbar kyphosis, and pectus carinatum [12]. At or around age 8, there is a concern for developing life-threatening airway obstruction due to tracheal deviation and narrowing [21-24]. This can result in shortness of breath and sleep apnea. The leading cause is an imbalance of the growth in trachea, bone, vessels, and other tissues. While the trachea and vessels continue to grow after age 10, the bone growth in most severe MPS IVA patients stops around age 10 - 11 because of deposits of GAG in the chondrocytes in the long bones, spine, ribs, and sternum and successive disruption of the growth plate region. The small upper thoracic cavity cannot accommodate the growing trachea and vessels, leading to tracheal impingement by the brachiocephalic artery, connective tissues and/or other tissues [25-26]. In addition, submucosal protrusions caused by the buckled tracheal wall pose difficulty in advancing the endotracheal tube to the mid-tracheal position. Reconstructive trachea surgery, which involves the removal of several redundant trachea segments, has been performed to address this life-threatening condition. Although this surgery has provided MPS IVA patients with dramatic respiratory improvements, it is a high risk, especially when patients present with SCC. Overall, the most common serious or life-threatening issues arise from 0 to 18 years in MPS IVA patients and will be required for ameliorating such signs and symptoms during the pediatric period.

Heart disease is common in patients with severe MPS IVA, although it may not develop or manifest until 2-3 decades in life. In severely affected MPS IVA patients, GAG storage causes heart valve thickening and regurgitation, impaired diastolic filling, and higher heart rates as patients age [27-28]. Typical findings include thickened and shortened mitral valve chordae, thickened, and rolled mitral valve edges, and fused aortic valve cusps. Left-sided valvular dysfunction, hypertrophy of the myocardium, intimal sclerosis of the coronary arteries, and vascular disease have developed in MPS IVA patients [24,27,29-32], leading to cardiomyopathy, cor pulmonale and/or death.

Clinical recognition of skeletal dysplasia is essential to diagnose MPS IVA, combined with radiographic, genetic, and biochemical tests. The clinical manifestations and resulting impaired mobility can reduce the patient's ability to perform activities of daily living (ADL) and may considerably impact the quality of life (QoL) of the patient and caregivers. The patient's QoL can be further compromised by frequent infections, impaired hearing, frequent surgeries, and (joint) pain and/or fatigue [33].

3.2 Proposed Indication and Use of AAV8-hGALNS

The proposed clinical use of AAV8-hGALNS is for the treatment of Mucopolysaccharidosis IVA, MPS IVA results from a deficiency of the GALNS enzyme due to deleterious variants in the GALNS gene. AAV8-hGALNS is an AAV8 vector expressing a functional human codon-optimized cDNA encoding GALNS under the control of the tissue-specific (liver) promoter. Data from MPS IVA mice treated with AAV vector suggested that starting at 4 weeks old allowed partial correction of bone pathology [34-35; see Table 1], suggesting that it is required to start treating at an early stage. MPS IVA patients with a severe phenotype typically stop growing around the age of 5 years old, while those with an attenuated phenotype may grow until their teenage years, but overall bone growth may vary by disease severity[1]. Clinical phenotypes of MPS IVA are widely ranged from the most severe form (less than 100 cm) to the attenuated form which reaches over 150 cm tall. Since the growth velocity declines after 2 years of age in a severe form of MPS IVA patients, early treatment is required for bone growth [20]. In young patients who are still growing, we anticipate an increase in final bone length, which aims to alleviate joint pain, gait struggles, and cramped abdominal space. We anticipate the therapy will prevent heart valve damage in both young and adult patients.

3.3 Reasons Why Such Therapy Is Needed

Patients with a severe form of MPS IVA, primarily related to cervical instability, heart valvular disease, and pulmonary and tracheal compromise, often do not survive beyond the second or third decade of life. Patients with mild manifestations of MPS IVA characterized by mild bone and visceral involvement have been reported to survive into the seventh decade of life [1,8,13,20,36-39]. Mortality and morbidity rates at the initial stage are primarily related to atlantoaxial instability and subsequent cervical myelopathy, which can cause bowel and bladder dysfunction and sleep apnea. Obstructive sleep apnea due to narrowing trachea can cause prolonged periods of hypoxia, pulmonary hypertension, and even death [2,23-24,40-42]. Airway obstruction also occurs secondary to the thickening of tissue in the upper airway due to mucopolysaccharide deposition. Patients with MPS IVA have a predisposition to pulmonary infection because of progressive truncal deformity and immobility, namely, restricted lung capacity. Early-onset coronary heart disease and valve thickening (aortic and mitral) with resultant cardiac dysfunction are described, and endocarditis prophylaxis is recommended. Corneal clouding can cause visual disturbance and photophobia. Enamel abnormalities predispose them to dental caries. With all these symptoms and morbidities, there are currently no effective therapies for skeletal dysplasia and heart disease in MPS IVA patients, and novel therapies are urgently needed to prevent irreversible disease complications and improve patient management.

The current standard of care for US MPS IVA patients includes surgical procedures and enzyme replacement therapy (ERT). ERT, elosulfase alpha, was approved for MPS IVA by the US Food and Drug Administration (FDA) in 2014 and is administered by a weekly intravenous infusion

over 5–6 hours [43-45]. The accumulated data indicate that ERT may reduce fatigue and improve patients' QoL, but it has not shown any impact on cartilage, bone pathology, or bone growth. Several groups have reported this by looking at the clearance of storage in the surgical remnants of patients undergoing ERT [46] and found no effect on bone growth improvement [22,47-48] nor on the reduction in surgical interventions in these patients [49]. Additionally, no reduction in the blood KS levels has been demonstrated after ERT [50-51]. Recent reports show ERT does not improve linear bone growth despite initiating treatment before 5 years (based on over 2 years of follow-up data) [22]. In addition, even prompt initiation of ERT (less than 2 months) has a limited effect on slowing the progression of the skeletal phenotype [48]. Furthermore, ERT infusion is associated with a short half-life (2 min in mice and 35 min in humans), suggesting that most infused enzymes do not reach the avascular cartilage [52-53]. Therefore, new therapeutic approaches are needed that target the skeletal system to address cartilage and bone pathology, the underlying cause of the most debilitating symptoms of MPS IVA [48].

While hematopoietic stem cell transplantation (HSCT) shows promise as it can target the bone and cartilage more effectively than ERT because of the continuous physiological level of GALNS expression, it does not apply to all patients because of limitations, such as the availability of a matched donor, the age limit for effective treatment, lack of well-trained staff, and the mortality risk of the procedure (e.g., graft-versus-host disease, infection). Thus, we hypothesize that AAV gene therapy can provide a supraphysiological level of long-term continuous enzyme circulation and address the challenges of ERT and/or HSCT.

The proposed approach will address the underlying cause of the disease.

In summary, current treatment options (ERT) only provide some symptomatic improvement (e.g., fatigue, QoL). An AAV gene therapy provides supraphysiological, long-term, continuous enzyme circulation and can target the underlying defect in the peripheral organs, bones, and cartilage, as was shown in nonclinical studies. Therefore, AAV gene therapy will address the underlying cause of the most debilitating symptoms of MPS IVA.

Treatment with AAV gene therapy in MPS IVA mice effectively improves bone, cartilage, and connective tissue pathology, valvular heart disease and normalizes blood KS. Thus, AAV gene therapy will provide immediate potential benefits, including clearance of excessive KS and C6S in circulation and improvement of ADL (endurance and respiratory function), and potential long-range benefits, including improvement of bone growth, valvular heart disease, hearing impairment, and laxity of joints.

4 DESCRIPTION OF AAV8-hGALNS AND SCIENTIFIC RATIONALE FOR USE

4.1 Description of AAV8-hGALNS

4.1.1 Drug Product Naming Convention

Adeno-Associated Virus 8 Human N-Acetylgalactosamine-6-sulfate sulfatase (AAV8-HGALNS)

4.1.2 Drug Product Physical, Chemical, and Pharmaceutical Properties

AAV8-hGALNS is an AAV8 vector expressing a functional human codon-optimized cDNA encoding *GALNS* under the control of *the liver-muscle tandem promoter*. AAV8 expressing a functional copy of hGALNS under the control of the tissue-specific (liver) promoter will be administered via a single IV infusion. Transduced cells will provide supraphysiological levels of enzyme activity in circulation, resulting in the systemic delivery of the enzyme into the target tissues, including bone, cartilage, connective tissues, and visceral organs, and successive improvement of critical symptoms, including skeletal dysplasia and multi-domains. This approach will allow cellular uptake by the mannose-6-phosphate receptor and localization to the lysosomes, promoting increased catabolism of KS and C6S in affected tissues and improving the signs and symptoms of the disease. A schematic of the vector transgene, a description of cassette features summarizing the salient features of the AAV vector, and the vector map is presented in Figure 2. The hGALNS nucleotide sequence is provided in Appendix 1.

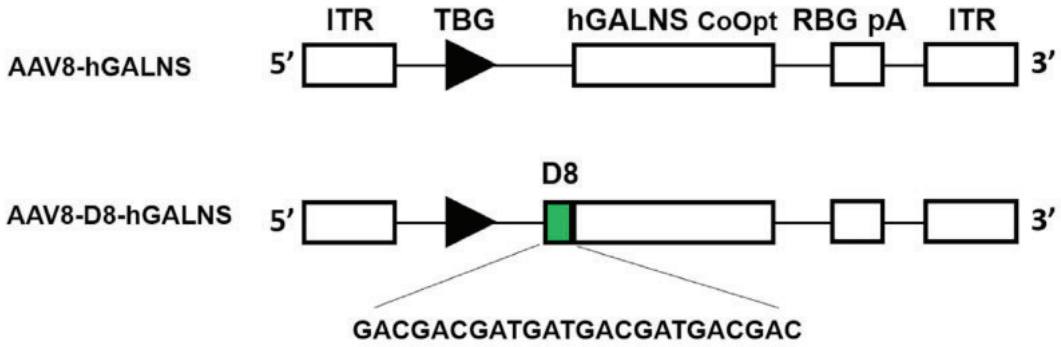

Figure 2. Vector Map

In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (for example, AAV8-ITRs), said hGALNS expression cassette comprising a nucleotide sequencing encoding a liver-specific promoter and a nucleotide sequence encoding hGALNS, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding hGALNS. In a specific embodiment, the liver specific promoter is a TBG promoter.

4.1.3 Drug Product Description, Mechanism of Action and Route of Administration

AAV8-hGALNS is a biologic modality for introducing corrected DNA into MPS IVA patients with biallelic pathogenic variants in the GALNS gene. The gene is delivered using an AAV virus, serotype AAV8, a modified virus that is not infectious in humans but maintains its natural ability to deliver genetic material into cells. The AAV8-hGALNS will introduce a normal copy of the human GALNS gene and help restore the normal function of the GALNS enzyme in the catabolism of KS, as shown in Figure 2. AAV8-hGALNS will be stored at -80°C and delivered to patients as an intravenous (IV) solution using the peripheral IV route of administration (ROA). The final formulation for the drug product will be finalized prior to IND. AAV8 tropism under a liver-related promoter will allow gene product expression mainly in liver.

4.2 Scientific Rationale for the Use of AAV8-hGALNS in the Rare Disease or Condition

MPS IVA is caused by biallelic pathogenic variants in the *GALNS* gene. It is caused by a deficiency of GALNS, a lysosomal enzyme involved primarily in the catabolism of glycosaminoglycans (GAGs). The mature active GALNS enzyme consists of 40- and 15-kDa subunits encoded by the *GALNS* gene [5,54-55]. GALNS cleaves the sulfates from the galactosyl residues in KS and the 6-sulfated *N*-acetylgalactosaminyl residue from C6S. Replacement of the GALNS enzyme through the delivery of AAV8-hGALNS gene therapy is expected to help normalize the metabolic defects associated with MPS IVA. AAV8 has a tropism for liver [56], especially under liver-specific promoter.

4.2.1 Clinical Efficacy of AAV8-hGALNS

To date, no clinical trial has been initiated with AAV8-hGALNS for treating patients with MPS IVA resulting from a deficiency of GALNS protein. However, as stated previously, MPS IVA patients who have undergone ERT or HSCT demonstrate a partial restoration of metabolic stability and improvement in stamina and endurance and, therefore, represent a clinical benchmark for gene replacement therapy that may lead to increased GALNS expression and activity, delivering the enzyme in the target organs and tissues. Clinical phenotypes [2,8], natural course of the disease [1,20,57], and therapeutic efficacy studies related to ERT and HSCT [41-42,47,58-62] were published. However, invasive studies were not conducted for the patients who had physical disability. Thus, there is a lack of longitudinal natural history for evaluating skeletal dysplasia and bone biomarkers and the broad population from birth to adulthood, as well as HSCT MPS IVA participants. Several publications indicated candidate biomarkers (KS and C6S) with outcomes corresponding to functioning and disease burden in skeletal dysplasia [48,63-65]. To fill in this gap, Nemours Children's Health, Delaware, initiated a Natural History Study (NHS) of the MPS IVA population. The non-invasive study is being conducted at the NCH-DV and has enrolled 60 participants ages 2 years and older. The cohort features a genetically heterogeneous MPS IVA population from various ethnic backgrounds, with 4 total visits occurring every 18 months. The study assesses outcome measures for clinical trials in an ongoing natural history study of MPS

IVA patients funded by the National Institutes of Health (NIH) (initiated May 1, 2021 - 1R01HD102545-01A1; Non-invasive functional assessment and pathogenesis of Morquio A-Protocol NCT05284006). Non-invasive testing includes BMD (Bone Mineral Density), pulmonary function test (PFT), magnetic resonance imaging (MRI), computed tomography (CT)/Angiogram, gait analysis, finger-joint laxity assessment, ADL/QOL questionnaire, and biomarkers related to the bone lesions.

4.2.2 Nonclinical Efficacy of AAV8-hGALNS

To understand the pathogenesis of the disease and to develop the experimental treatment for MPS IVA, three MPS IVA (knock-out, knock-in, human GALNS tolerant) mouse models have been established [66-68; see Table 1]. The phenotype and the pathophysiology of these mice have been well characterized.

Knock-out Galns$^{-/-}$ mice (MKC):
Homozygous Galns$^{-/-}$ mice have no detectable GALNS enzyme activity and show increased urinary GAG levels. These mice accumulate GAGs in multiple tissues, including liver, kidney, spleen, heart, brain, and bone marrow, and represent a mild MPS IVA phenotype. In 2-month-old mice, lysosomal storage is present primarily within reticuloendothelial cells such as Kupffer cells and cells of the sinusoidal lining of the spleen. Additionally, at 12 months, vacuolar change is observed in the visceral epithelial cells of glomeruli and cells at the base of heart valves, but it is not present in parenchymal cells such as hepatocytes and renal tubular epithelial cells. Hippocampal and neocortical neurons and meningeal cells had lysosomal storage in the brain. Radiographs revealed no change in the skeletal bones of mice up to 12 months old. This murine model shows visceral storage of GAGs but lacks skeletal features [66].

Human GALNS tolerant Galns$^{tm(hC79S\ mC76S)slu}$ mice (Mtol):
The MPS IV mouse (Galns$^{tm(hC79S\ mC76S)slu}$) model, which has many similarities to a mild phenotype of human MPS IVA, is a good model to evaluate long-term administration of enzyme replacement. It is tolerant to human GALNS protein. At 3 months, lysosomal storage is marked within hepatocytes, reticuloendothelial Kupffer cells, and cells of the sinusoidal lining of the spleen, neurons, and meningeal cells. The storage in bone is also obvious, with lysosomal distention in osteoblasts and osteocytes lining the cortical bone, chondrocytes, and sinus-lining cells in bone marrow. Ubiquitous expression of the inactive human GALNS was confirmed by western blot [7]. In the Galns $^{tm(hC79S\ mC76S)slu}$ model, the enzyme activities of other sulfatases was substantially reduced [67].

To assess the efficacy of AAV gene therapy, two mouse models of MPS IVA have been used: Knock-out **Galns$^{-/-}$ (MKC) and human GALNS tolerant Galns$^{tm(hC79S\cdot mC76S)slu}$ (Mtol)** mouse models.

Non-GLP studies have been carried out at the NCH-DE Skeletal Dysplasia Lab at the Dupont Experimental Station. These studies support the use of AAV8 expressing a functional copy of hGALNS under the control of various promoters (Table 1).

Table 1: Summary of Preclinical studies on AAV gene therapy for MPS IVA

Study	Model	Treatment conditions	Vector/Promoter	Results
Study 1: published POC	MKC and Mtol mouse model	Treated at 4 weeks of age, both male and female were treated with i.v. injections	AAV8 vector packaged and hGALNS expressing construct under TBG promoter (liver-specific thyroxine-binding globulin)	•Dose $5x10^{13}$ vg/kg •Resulted in supraphysiological levels of enzyme activity in plasma and reduced KS levels in plasma to normal levels at two weeks post-injection. •Detected GALNS activity in target tissues, including bone and heart, improvements were detected in bone and heart pathology
Study 2: published	MKC mouse model	Treated at 4 weeks of age, both male and female were treated with i.v. injections	AAV8 vector packaging an hGALNS expressing construct under TBG (liver-specific thyroxine-binding globulin) promoter or ubiquitous (CAG) promoter	•Dose $5x10^{13}$ and $2x10^{14}$ vg/kg •Resulted in supraphysiological levels of enzyme activity in plasma and was maintained during 12 weeks post AAV delivery in male mice •Female mice had comparatively lower plasma enzyme activity levels, but the lower levels were maintained •Dose dependent elevation of enzyme activity was observed in KO mice with both constructs but higher activity in bone was detected from CAG promoter •Reduction of KS levels in plasma and liver with both vectors
Study 3: unpublishe+F+A11	MKC and Mtol mouse model	Treated at 4 weeks of age, both male and female were treated with i.v. injections	AAV8 vector packaging an hGALNS expressing construct under TBG (liver-specific thyroxin-biding globulin) promoter or ubiquitous (CAG) promoter	•Dose $5x10^{13}$ and $2x10^{14}$ vg/kg •Supraphysiological levels of enzyme activity and reduced KS levels in plasma were maintained for 24 weeks in the male mice. •Higher enzyme activity and higher impact on bone lesions were detected from CAG promoter
Study 4: unpublished	MKC mouse model	Treated at 4 weeks of age, both male and female were treated with i.v. injections	AAV8 and AAV9 vectors with better optimizing expression cassettes containing CpG-depleted hGALNS complementary deoxyribonucleic acid (cDNA) and new promoters (Liver muscle tandem promoter (LMTP) and liver-bone tandem promoter (LBTP) in comparison with CAG and TBG	•Dose $5x10^{13}$ vg/kg •All the vectors comparable in the enzyme plasma activity and their ability to reduce the KS levels in plasma, liver and other peripheral organs •Only CAG (AAV8) and LMTP (AAV9) provided supraphysiological levels of enzyme activity in bone •Initial evidence suggest that LMTP promoter is comparable to CAG and better than TBG •LMTP (AAV9) produced higher enzyme activities in bone and muscle than the other promoters

These completed studies with the vectors tested demonstrate a clear benefit resulting in sustained enzyme activity, substrate reduction, and improvement in heart valve, growth plate, and cartilage pathology following gene therapy. These results suggest that the continuous presence of high levels of circulating enzyme increases cartilage and heart valve penetration and reduces the KS level, thereby improving GAG storage in these regions. This has not been observed in mouse ERT studies [52]. These data also demonstrate that the expression construct can produce biologically active protein, i.e., the enzyme expressed can degrade GAGs in the target tissues.

As the transgene product is a secreted enzyme and intends to have a therapeutic effect beyond the cells expressing it, per cell expression has not been explored. However, histochemical evaluations are ongoing to examine individual cell expression levels. As described above (Study 1), supraphysiological levels of the enzyme have been demonstrated in circulation and the liver, resulting in the normalization of GAG levels in plasma and a substantial reduction of GAG levels in the periphery. The vector biodistribution analyses showed robust transduction of liver [KO (AAV-TBG-hGALNS) 242.23 ± 114.08 (n = 7), KO (AAV-TBG-D8-hGALNS) 182.36 ± 71.85 (n = 6), Mtol (AAV-TBG-hGALNS) 309.51 ± 112.14 (n = 5), Mtol (AAV-TBG-D8- hGALNS) 305.36 ± 147.82 (n = 4) in AAV vector copies per diploid genome)], which was sufficient to correct pathology in liver, heart, and other peripheral organs as well as improvement in cartilage and bone (Figs. 3 and 4; Table 2).

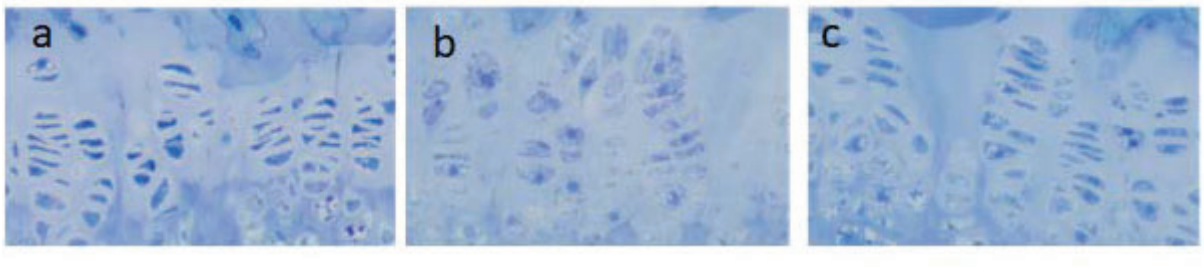

Figure 3. Correction of bone pathology in MPS IVA mice treated with AAV8 vectors. Correction of chondrocytes vacuolization was assessed by toluidine blue staining analysis using light microscopy of growth plate in the knee joint of MPS IVA mice treated with AAV8 vectors. Bone pathology in tolerant (MTOL) mice was compared with a) wild-type, b) untreated MPS IVA and c) treated MPS IVA with AAV8 vectors [34].

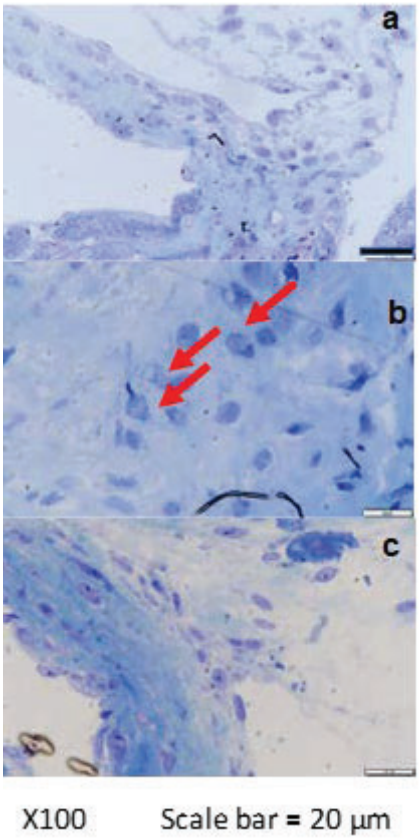

Figure 4. Correction of heart pathology in MPS IVA mice treated with AAV8 vectors. Correction of vacuolization was assessed by toluidine blue staining analysis using light microscopy of heart valve of MPS IVA mice treated with AAV8 vectors. Heart pathology in tolerant (MTOL) mice were compared with wild-type, untreated MPS IVA, and treated MPS IVA with AAV8 vectors. The arrows indicate the location of disease-related vacuoles. a) wild-type (WT), b) untreated MTOL, c) MTOL with AAV8-hGALNS.

Table 2: AAV vector biodistribution in liver

Vector	Mouse Model	Biodistribution (AAV vector copies per diploid genome)
AAV-TBG-hGALNS	MPS IV A KO	242.23 ± 114.08 (n = 7)
	Mtol	309.51 ± 112.14 (n = 5)
AAV-TBG-D8-hGALNS	MPS IV A KO	182.36 ± 71.85 (n = 6)
	Mtol	305.36 ± 147.82 (n = 4)

No toxicity or severe immune response was observed in POC studies using MKC mice at doses as high as 2 x 10^{14} vg/kg. In addition, treated MKC mice (5 x 10^{13} vg/kg) have been observed up to

48 weeks post-AAV gene therapy, and no toxicity or tumorigenesis has been observed. All KO mice in studies developed antibodies against the hGALNS enzyme expressed from the AAV vector, like the available data on elosulfase alpha ERT in rats and monkeys [69]. No significant correlation between antibody levels and enzyme activity or substrate reduction was observed in the study [34-35]. In the Mtol mice, the development of antibodies to the expressed enzyme was minimal to none, indicating that some aspect of the antibody response is because of the complete absence of the enzyme and expressing a human enzyme in a mouse.

In addition, no AEs have been observed from a nonuniform transgene expression within the target population. Furthermore, nonuniform expression is unlikely as this is a secreted lysosomal enzyme that can cross-correct neighboring cells.

Therapeutic Expression Construct

The proposed therapeutic expression construct has been rationally designed and evaluated to support its use in this population. Ubiquitous and tissue-specific promoters have been evaluated in *in vivo* models. Liver-specific or liver and bone/muscle promoters were similarly effective compared to ubiquitous promoters. Therefore, an expression cassette with a liver-specific promoter driving the expression of a codon-optimized hGALNS cDNA was selected the TBG promoter was used clinically.

Immunogenic potential of transgene protein

As most MPS IVA patients begin ERT as soon as they are diagnosed, patients are not naïve to the expressed protein. The development of antibodies (anti-GALNS Ab) is common among patients on ERT, irrespective of the genotype. These patients are not routinely on immunosuppressive regimens, and no clinical symptoms have been reported. Data supporting the approval of ERT showed anti-GALNS Ab in rat studies, which could be because of the human protein in a rodent model. The similar level of anti-GALNS Ab was also observed in the mouse studies with AAV gene therapy, more pronounced in the KO model and negligible in the Mtol model, which expresses a non-functional hGALNS. The need for an immunosuppressive regimen incorporated into the protocol is due to the systemic use of AAV vectors.

Vector capsid

The vector biodistribution has been characterized in peripheral tissues in mouse models of MPS IVA. The proposed capsid is AAV8, and the biodistribution of AAV8 delivered via the IV route has been reported in rodents and large animal models [70-73] and in human clinical trials for multiple doses [74-77]. The potential for germline transduction of the capsid has been evaluated in MPS IVA mice treated with AAV vectors. There was no evidence of an increase in the copy number of the vector in ovary and testis compared with other tissues (muscle, bone, and liver). Additionally, this analysis is recommended in the dose-ranging study to be conducted before the clinical trial. Negligible recombinant AAV transduction in germline cells has been reported based on several studies in non-human primates and other disease models [70,78].

Dose translation strategy

A dose-ranging study is needed to identify clinical dosing. Based on the POC studies, the expected range is 5 x 10^{13} vg/kg - 1 x 10^{14} vg/kg. To provide a rough estimate, the anticipated total vector required for a 3-year-old patient weighing around 14.5 kg [mean weights for a 3-year-old girl (13.9 kg) and boy (14.4 kg)] is around 1.5 x 10^{15} vector genomes. The dose to be used in the clinical trial will be chosen based on improved bone/cartilage pathology, KS levels in plasma, and enzymatic activity in plasma. A minimum efficacious and anticipated maximum efficacious dose has not yet been established in nonclinical studies. At both doses tested, supraphysiological enzymatic activity in plasma, and normalization of KS levels were observed. No dose-dependent difference was observed. Therefore, 5 x 10^{13} vg/kg or less dose will be used clinically.

Non-clinical plan to address known toxicities associated with AAV gene therapies

Significant effort has been undertaken or planned in nonclinical studies to address the known toxicities associated with AAV gene therapies. Regarding dorsal root ganglion (DRG) toxicity, mouse studies have not evaluated DRG toxicity. While AAV8 capsid distribution can be analyzed in the DRGs, the tissue-specific promoter proposed will not be expressed in DRGs. Therefore, it is highly unlikely to have expression related to DRG toxicity. However, this issue is to be explored in the dose-ranging study, which will be required to determine the clinical doses for this study.

Hepatomegaly or any obvious liver toxicity phenotype was not observed in the mouse studies with both short-term (12 weeks post AAV delivery) and long-term (24- and 48-weeks post AAV delivery) cohorts. Liver enzyme levels [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] were normal in these mice post-treatment. In addition, this issue is to be explored in the dose-ranging study.

Complement-related toxicities have not been evaluated in mouse studies. Even at a relatively high dose of 2 x 10^{14} vg/kg, the mice had no observable toxicity. However, this issue is to be explored in the dose-ranging study.

Cell-mediated immunity to the capsid or transgene leading to loss of transduced cells has not been evaluated in mouse studies. In the dose-ranging studies, we propose to do ELISpots to look for the transgene and/or capsid-specific T-cell immune responses.

5 ORPHAN DRUG STATUS

AAV8-hGALNS is not designated as an orphan drug or product for the treatment of patients with MPS IVA resulting from a deficiency of GALNS, and AAV8-hGALNS is currently not designated as an orphan drug or product in the United States for any other indication.

Previous Orphan Drug Designations (ODDs) for MPS IVA have been approved for lysosomal enzyme N-acetylgalactosamine-6-sulfate sulfatase in 2008 and elosulfase alfa in 2009, but there is still an unmet need for this patient population regarding effective treatment options, given the

rarity of the disease and the small patient population size. Currently, the available treatment and disease management options fail to address the bone growth that is lost at an early age in MPS IVA patients, which leads to a lot of the ongoing symptoms and morbidities faced by this population.

6 PATIENT SUBSET CONSIDERATIONS AND MEDICAL PLAUSIBILITY OF THE CHOSEN SUBSET

The prevalence of MPS IVA does not exceed 200,000. Therefore, subset considerations and medical plausibility of a subset are not applicable to the proposed indication of patients with MPS IVA resulting from a deficiency of GALNS in this request.

7 REGULATORY STATUS AND MARKETING HISTORY

NIH is currently in late phase pre-clinical development of AAV8-hGALNS and a pIND meeting is planned with FDA, Center for Biologics Evaluation and Research (CBER), and Office of Therapeutic Products (OTP) to discuss our completed proof of concept (POC) studies and planned IND enabling toxicology studies in preparation for IND submission estimated for 2026.

AAV8-hGALNS is not currently marketed or approved for use in any country, including the United States. In accordance with section 526(a)(1) of the Federal Food, Drug, and Cosmetic Act [21 USC 360bb(a)(1)]; 21 CFR 316.20(b)(7); and 21 CFR 316.23(a), NCATS has not previously submitted a marketing application to FDA for the same active moiety in AAV8-hGALNS for the same rare disease or condition prior to submission of the AAV8-hGALNS orphan drug designation request.

8 DOCUMENTATION OF PATIENT POPULATION SIZE

8.1 Prevalence of the orphan disease or condition in the United States

Documentation of the number of MPS IVA-affected people in the US is scarce within the context of orphan drug application in the US [3].

There are a few unique sources of information on the prevalence of MPS IVA worldwide [79-80], and clinical data from various counties with similar populations is indicative of those who form ethnic communities within the US. Therefore, data published in the literature were used to derive prevalence estimates for MPS IVA in the United States based on the US population and estimated births per 1,000 people according to FDA guidelines for orphan drug applications.

Methods:

A search of bibliographic data was performed on 24 March 2024.

Data Source: www.pubmed.gov

Search terms: MPS IVA, Morquio A, Prevalence, Incidence
Languages: All

Time period included: Until 24 March 2024 without limitation.

Results:

The published data regarding epidemiological information were compiled (Appendix 2). As limited publications have been retrieved, all data, i.e., also data from countries outside the United States, have been included in the overview. Our extensive database searches have not identified any data published about the prevalence of MPS IVA in the United States. The early data from other countries show that MPS IVA is a rare disease with a reported birth incidence of 1 per 200,000 live births in British Colombia [81-82]. Although the population of British Columbia is small compared to the US, its mixture of immigrants from Europe and Asia mirrors that found in the US. Prevalence is not expected to differ remarkably between the United States and other countries.

Patients with a severe phenotype accounted for around 70% of the total MPS IVA patients registered in the International Morquio Registry [1]. The current maximum survival of infants with severe MPS IVA is between 20-30 years of age. According to a retrospective study conducted in the US between 2015 and 2024, there is a 12.0 births/1000 people birth rate, which in the US population of 341,814,420 in 2024 provides for an estimated 4,101,773 total live births per year [3]. The actual number of births was essentially unchanged between 2020 and 2022 (3,613,647, 3,664,292, and 3,667,758; on average, 3,648,565 births per year) [1]. Based on the incidence of MPS IVA in the European countries (Portugal, Germany, Northern Ireland, Holland, as well as Australia and Canada, populated by immigrants from Europe), we calculate an incidence in live births of about 1/200,000 (See Section 8.2). This would result in an annual 18.2 estimated number of live births of infants in the US with MPS IVA. Even if 100% of these afflicted infants survived to their thirtieth (30^{th}) birthday, this would have resulted in a theoretical total of 547 afflicted patients accumulated over the 30 years in the US.

In the United States, 98 cases have been registered in the International Morquio A Registry [1]. Assuming mild cases and some severe cases have not been registered, roughly 250 cases of patients with severe and mild disease can be estimated to exist in the US annually. This number of estimated patients is approximately 800-fold lower than the prevalence criterion (<200,000 people affected), and if the total of 547 patients is used, a 365-fold number is obtained; both these values are well below the prevalence rates defined by the FDA for eligibility for orphan drug designation.

Based on the MPS IVA patient registration data from the UK MPS Society discussed below in more detail, the UK data corrected for 5-fold larger US populations would give an estimated total number of patients at 455.

The best registry of MPS IVA patients is in The United Kingdom, where there is a 100% registration of such patients through the UK MPS Society. Note that the UK population as of July

2007 is 60,777,238 people; thus, the total number of patients registered per year is shown below. Twenty-one patients were diagnosed during 1990-2003 (12 years), and the average number of diagnosed patients was 3.17 per year. Since the US estimated total live births is estimated to be 6.19-fold higher than that in the UK [an estimated 695,500 total live births per year (est. 2003)], the estimated number of MPS IVA patients in the US would be 19.62 per year. Based on the below data, 40-50% of the UK registered MPS IVA patients died by their twenties (1978 -1989). Thus, based on the UK survival data, one could estimate that there would be 455 patients in the US during a similar period, of which 40-50% would die within the third decade. Those patients who died were deemed as severe phenotypes.

In our clinic at NCH-DE, 178 MPS IVA patients have been registered in our system and are indicated as having a Morquio A Syndrome diagnosis.

8.2 Prevalence of the orphan disease or condition in other countries, with Immigrant Populations in the U.S.

Results:
MPS IVA is a rare disease with a reported birth incidence in European countries of 1 per 76,320 live births in Northern Ireland, based on ascertained cases over 30 years [83], 1 in 167,000 live births in Portugal for 20 years [84], 1 in 263,000 live births in Germany for 1980 to 1995 [85], and 1 in 450,000 live births in The Netherlands for 26 years (1970-1996) [86].

Table 3 Live Birth Incidence of MPS IVA in countries of the USA and EU

Country	Live Birth Incidence	Alive prevalence	Covered Period	Source
USA	0.11/100,000	0.03/100,000	1995-2015	Puckett et al. 2021 [3]
Czech Republic	0.73/100,000	0.71/100,000	1975–2008	Poupětová et al. 2010 [87]
Denmark*	0.48/100,000	0.31/100,000	1975–2004	Malm et al. 2008 [88]
Germany	0.38/100,000	N/A	1980-1995	Baehner et al. 2005 [85]
Northern Ireland	1.31/100,000	0.86/100,000	1958-1985	Nelson 1997 [89]
Norway*	0.76/100,000	0.24/100,000	1979 –2004	Malm et al. 2008 [88]
Poland*	0.14/100,000	N/A	1970 –2010	Jurecka et al. 2015 [90]
Portugal	N/A	0.6/100,000	1982 –2001	Pinto et al. 2004 [84]
Sweden*	0.07/100,000	0.07/100,000	1975 –2004	Malm et al. 2008 [88]

Switzerland*	0.38/100,000	N/A	1975 –2008	Khan et al. 2018 [91]
The Netherlands	0.36/100,000	0.22/100,000	1970-1996	Poorthuis et al. 1999 [86]

*Represent all MPS IV cases (A and B).

The following data were published for countries outside the United States and the EU: The British Columbia survey reported the incidence of MPS IV to be 1 per 200,000 live births [81-82] (Applegarth et al. 2000; Lowry et al. 1990). In the Australian population, the estimated incidence was 1 in 201,000 live births and the prevalence 1 in 169,000 [92]. In Western Australia, the birth incidence was determined to be 1 per 640,000 live births [83]. In Japan, the incidence was 1 per 667,000 live births.

Table 4 Live Birth Incidence of MPS IVA in countries outside the United States and the EU

Country	Live Birth Incidence	Alive prevalence	Covered Period	Source
Australia	N/A	0.59/100,000	1980-1996	Meikle et al. 1999 [92]
Western Australia	0.15/100,000	N/A	1969-1996	Nelson et al. 2003 [83]
Canada (British Columbia)	1.9/100,000	0.48/100,000	1972-1996	Applegarth et al. 2000 [81]
Brazil	0.15/100,000	N/A	1982-2019	Josahkian et al. 2020 [93]
Columbia*	0.68/100,000	N/A	1998-2007	Gomez et al. 2012 [94]
Mexico	1.1/100,000	N/A	2012-2017	Mendoza-Ruvalcaba et al. 2020 [95]
Taiwan	0.33/100,000	N/A	1984-2004	Lin et al. 2009 [96]
Japan*	N/A	0.15/100,000	1982-2009	Khan et al. 2017 [91]
Saudi Arabia*	3.6/100,000	N/A	1983-2008	Moammar et al. 2010 [97]
South Korea*	0.13/100,000	N/A	1994-2013	Cho et al. 2014 [98]
Tunisia*	0.86/100,000	0.68/100,000	1999-2021	Nasrallah et al. 2023 [99]

*Represent all MPS IV cases (A and B).

Discussion:

MPS IVA seems to be more common in Northern Ireland than in other countries. Nelson [16,89] mentioned marked heterogeneity in the clinical presentation of the patients. It is also probable that some epidemiological studies comprise severe cases of MPS IVA and that false negative screening tests might have caused an underestimation of the live birth incidence. Tomatsu (1995) [68] identified a common missense mutation in severe MPS IVA patients on Irish background, indicating the high incidence in Northern Ireland derived from the common founder effect. Furthermore, variations in the frequency of specific mutations in different countries might have contributed to varying incidence rates. Data published for Greece [100] and Turkey [101] have not been related to demographic data. Therefore, these studies were only listed in Appendix 2.

Conclusions

- A search of bibliographic data was used to derive estimates of MPS IVA-affected people according to FDA guidelines for orphan drug applications.
- Prevalence data from the United States are scarce and remain uncertain.
- The live birth incidence in European countries was reported to be between 1/450,000 and 1/76,320, corresponding to 0.022 per 10,000 live births and 0.131 per 10,000 live births.
- Even on the assumption that mild cases were neither diagnosed nor registered, the evaluation of the rare epidemiological data shows that MPS IVA clearly meets the criterion (< 200,000 people affected) defined by the FDA for orphan drug application.

9 RARE PEDIATRIC DISEASE DESIGNATION JUSTIFICATION

Based on the evidence presented above, pursuant to Section 908 of FDASIA, and consistent with the Rare Pediatric Disease Priority Review Vouchers Guidance for Industry, we believe that AAV8-hGALNS qualifies as a Rare Pediatric Drug Product for the treatment of GALNS-related MPS IVA. Specifically:

1) MPS IVA is a serious or life-threatening disease in which the serious or life-threatening manifestations primarily affect individuals aged from birth to 18 years. Most MPS IVA patients are identified at birth through newborn screening (NBS). Despite this early recognition and initiation of best-available therapies, including ERT, and severely affected patients continue to experience serious or life-threatening complications of their disease with various surgical interventions. The majority of patients will manifest serious progressive skeletal dysplasia, such as marked short stature with short neck, spinal cord compression and instability of neck, obstructive airway and restrictive lung, kyphoscoliosis, hip dysplasia and dislocation, and knock-knee during childhood and adolescence, and there is a need for better treatments to prevent, mitigate or ameliorate these disease-related complications.

2) GALNS-related MPS IVA is a rare disease, with an estimated prevalence of fewer than 600 patients in the US (see discussion in Section 8).

Additionally, we note that AAV8-hGALNS is intended as a gene therapy specifically for gene replacement due to mutations in the GALNS enzyme and has no foreseeable use in the treatment of any other disease. AAV8-hGALNS is currently in the late pre-clinical phases of testing at NCH-

DV and NCATS, with planned progression to clinical trials within the next few years. The most likely patient population will be children or adolescents.

We therefore request that AAV8-hGALNS be designated as a Rare Pediatric Disease Product for the treatment of the GALNS-related MPS IVA.

10 REFERENCES

1. Montaño AM, Tomatsu S, Gottesman GS, Smith M, Orii T. International Morquio A Registry: clinical manifestation and natural course of Morquio A disease. J Inherit Metab Dis. 2007 Apr;30(2):165-74. doi:10.1007/s10545-007-0529-7. Epub 2007 Mar 8. PMID: 17347914.
2. Lavery C, Hendriksz C. Mortality in patients with morquio syndrome a. JIMD Rep. 2015;15:59-66. doi:10.1007/8904_2014_298. Epub 2014 Apr 10. PMID: 24718838; PMCID: PMC4270860.
3. Puckett Y, Mallorga-Hernández A, Montaño AM. Epidemiology of mucopolysaccharidoses (MPS) in United States: challenges and opportunities. Orphanet J Rare Dis. 2021 May 29;16(1):241. doi:10.1186/s13023-021-01880-8. PMID: 34051828; PMCID: PMC8164808.
4. Baker E, Guo XH, Orsborn AM, Sutherland GR, Callen DF, Hopwood JJ, Morris CP. The morquio A syndrome (mucopolysaccharidosis IVA) gene maps to 16q24.3. Am J Hum Genet. 1993 Jan;52(1):96-8. PMID: 8434612; PMCID: PMC1682100.
5. Tomatsu S, Fukuda S, Masue M, Sukegawa K, Fukao T, Yamagishi A, Hori T, Iwata H, Ogawa T, Nakashima Y, et al. Morquio disease: isolation, characterization and expression of full-length cDNA for human N-acetylgalactosamine-6-sulfate sulfatase. Biochem Biophys Res Commun. 1991 Dec 16;181(2):677-83. doi:10.1016/0006-291x(91)91244-7. PMID: 1755850.
6. Leal AF, Alméciga-Díaz CJ, Tomatsu S. Mucopolysaccharidosis IVA: Current Disease Models and Drawbacks. Int J Mol Sci. 2023 Nov 9;24(22):16148. doi: 10.3390/ijms242216148. PMID: 38003337; PMCID: PMC10671113.
7. Tomatsu S, Montaño AM, Nishioka T, Gutierrez MA, Peña OM, Tranda Firescu GG, Lopez P, Yamaguchi S, Noguchi A, Orii T. Mutation and polymorphism spectrum of the GALNS gene in mucopolysaccharidosis IVA (Morquio A). Hum Mutat. 2005 Dec;26(6):500-12. doi: 10.1002/humu.20257. PMID: 16287098.
8. Hendriksz CJ, Harmatz P, Beck M, Jones S, Wood T, Lachman R, Gravance CG, Orii T, Tomatsu S. Review of clinical presentation and diagnosis of mucopolysaccharidosis IVA. Mol Genet Metab. 2013 Sep-Oct;110(1-2):54-64. doi: 10.1016/j.ymgme.2013.04.002. Epub 2013 Apr 10. PMID: 23665161; PMCID: PMC3755102.
9. Verheyen S, Blatterer J, Speicher MR, Bhavani GS, Boons GJ, Ilse MB, Andrae D, Sproß J, Vaz FM, Kircher SG, Posch-Pertl L, Baumgartner D, Lübke T, Shah H, Al Kaissi A, Girisha KM, Plecko B. Novel subtype of mucopolysaccharidosis caused by arylsulfatase K (ARSK) deficiency. J Med Genet. 2022 Oct;59(10):957-964. doi: 10.1136/jmedgenet-2021-108061. Epub 2021 Dec 16. PMID: 34916232; PMCID: PMC9554054.
10. Funderburgh JL. Keratan sulfate: structure, biosynthesis, and function. Glycobiology. 2000 Oct;10(10):951-8. doi: 10.1093/glycob/10.10.951. PMID: 11030741.
11. Kornfeld S, Mellman I. The biogenesis of lysosomes. Annu Rev Cell Biol. 1989;5:483-525. doi: 10.1146/annurev.cb.05.110189.002411. PMID: 2557062.
12. Tomatsu S, Montaño AM, Oikawa H, Giugliani R, Harmatz P, Smith M, et al. Impairment of body growth in mucopolysaccharidoses. Handbook of Growth and Growth Monitoring in Health and Disease. 2011 Oct 25;2091–117. doi:10.1007/978-1-4419-1795-9_126

13. Tomatsu S, Montaño AM, Oikawa H, Smith M, Barrera L, Chinen Y, Thacker MM, Mackenzie WG, Suzuki Y, Orii T. Mucopolysaccharidosis type IVA (Morquio A disease): clinical review and current treatment. Curr Pharm Biotechnol. 2011 Jun;12(6):931-45. doi: 10.2174/138920111795542615. PMID: 21506915.

14. Northover H, Cowie RA, Wraith JE. Mucopolysaccharidosis type IVA (Morquio syndrome): a clinical review. J Inherit Metab Dis. 1996;19(3):357-65. doi: 10.1007/BF01799267. PMID: 8803780. ***Literature copy not provided.***

15. Wraith JE. The mucopolysaccharidoses: a clinical review and guide to management. Arch Dis Child. 1995 Mar;72(3):263-7. doi: 10.1136/adc.72.3.263. PMID: 7741581; PMCID: PMC1511064.

16. Nelson J, Thomas PS. Clinical findings in 12 patients with MPS IV A (Morquio's disease). Further evidence for heterogeneity. Part III: Odontoid dysplasia. Clin Genet. 1988 Feb;33(2):126-30. doi: 10.1111/j.1399-0004.1988.tb03423.x. PMID: 3129223.

17. Fukuda S, Tomatsu S, Masue M, Sukegawa K, Iwata H, Ogawa T, Nakashima Y, Hori T, Yamagishi A, Hanyu Y, et al. Mucopolysaccharidosis type IVA. N-acetylgalactosamine-6-sulfate sulfatase exonic point mutations in classical Morquio and mild cases. J Clin Invest. 1992 Sep;90(3):1049-53. doi:10.1172/JCI115919. PMID: 1522213; PMCID: PMC329963.

18. Orii T, Minami R, Chiba T, et al. Study on Morquio syndrome. Bone Metab. 1971;5(1): 72–78. (in Japanese). ***Literature copy not provided.***

19. Beck M, Glössl J, Grubisic A, Spranger J. Heterogeneity of Morquio disease. Clin Genet. 1986 Apr;29(4):325-31. doi: 10.1111/j.1399-0004.1986.tb01262.x. PMID: 3087664.

20. Montaño AM, Tomatsu S, Brusius A, Smith M, Orii T. Growth charts for patients affected with Morquio A disease. Am J Med Genet A. 2008 May 15;146A(10):1286-95. doi: 10.1002/ajmg.a.32281. PMID: 18412124.

21. Averill LW, Kecskemethy HH, Theroux MC, Mackenzie WG, Pizarro C, Bober MB, Ditro CP, Tomatsu S. Tracheal narrowing in children and adults with mucopolysaccharidosis type IVA: evaluation with computed tomography angiography. Pediatr Radiol. 2021 Jun;51(7):1202-1213. doi: 10.1007/s00247-020-04946-0. Epub 2021 Feb 20. PMID: 33608742; PMCID: PMC10190165.

22. Doherty C, Stapleton M, Piechnik M, Mason RW, Mackenzie WG, Yamaguchi S, Kobayashi H, Suzuki Y, Tomatsu S. Effect of enzyme replacement therapy on the growth of patients with Morquio A. J Hum Genet. 2019 Jul;64(7):625-635. doi: 10.1038/s10038-019-0604-6. Epub 2019 Apr 24. PMID: 31019230.

23. Pizarro C, Davies RR, Theroux M, Spurrier EA, Averill LW, Tomatsu S. Surgical Reconstruction for Severe Tracheal Obstruction in Morquio A Syndrome. Ann Thorac Surg. 2016 Oct;102(4):e329-31. doi: 10.1016/j.athoracsur.2016.02.113. PMID: 27645974.

24. Tomatsu S, Averill LW, Sawamoto K, Mackenzie WG, Bober MB, Pizarro C, Goff CJ, Xie L, Orii T, Theroux M. Obstructive airway in Morquio A syndrome, the past, the present and the future. Mol Genet Metab. 2016 Feb;117(2):150-6. doi: 10.1016/j.ymgme.2015.09.007. Epub 2015 Sep 21. PMID: 26432669; PMCID: PMC4755902.

25. Shinhar SY, Zablocki H, Madgy DN. Airway management in mucopolysaccharide storage disorders. Arch Otolaryngol Head Neck Surg. 2004 Feb;130(2):233-7. doi: 10.1001/archotol.130.2.233. PMID: 14967758.

26. Pelley CJ, Kwo J, Hess DR. Tracheomalacia in an adult with respiratory failure and Morquio syndrome. Respir Care. 2007 Mar;52(3):278-82. PMID: 17328826.

27. Kampmann C, Abu-Tair T, Gökce S, Lampe C, Reinke J, Mengel E, Hennermann JB, Wiethoff CM. Heart and Cardiovascular Involvement in Patients with Mucopolysaccharidosis Type IVA (Morquio-A Syndrome). PLoS One. 2016 Sep 9;11(9):e0162612. doi: 10.1371/journal.pone.0162612. PMID: 27610627; PMCID: PMC5017658.

28. Leong HY, Abdul Azize NA, Chew HB, Keng WT, Thong MK, Mohd Khalid MKN, Hung LC, Mohamed Zainudin N, Ramlee A, Md Haniffa MA, Yakob Y, Ngu LH. Clinical, biochemical and genetic profiles of patients with mucopolysaccharidosis type IVA (Morquio A syndrome) in Malaysia: the first national natural history cohort study. Orphanet J Rare Dis. 2019 Jun 14;14(1):143. doi:10.1186/s13023-019-1105-6. PMID: 31200731; PMCID: PMC6570902.

29. Ireland MA, Rowlands DB. Mucopolysaccharidosis type IV as a cause of mitral stenosis in an adult. Br Heart J. 1981 Jul;46(1):113-5. doi:10.1136/hrt.46.1.113. PMID: 6791672; PMCID: PMC482614.

30. John RM, Hunter D, Swanton RH. Echocardiographic abnormalities in type IV mucopolysaccharidosis. Arch Dis Child. 1990 Jul;65(7):746-9. doi:10.1136/adc.65.7.746. PMID: 2117422; PMCID: PMC1792426.

31. Factor SM, Biempica L, Goldfischer S. Coronary intimal sclerosis in Morquio's syndrome. Virchows Arch A Pathol Anat Histol. 1978 Aug 4;379(1):1-10. doi:10.1007/BF00432778. PMID: 150685. ***Literature copy not provided.***

32. Engle J, Safi HJ, Abbassi O, Iliopoulos DC, Dorsay D, Cartwright J Jr, Weilbaecher D. Mucopolysaccharidosis presenting as pediatric multiple aortic aneurysm: first reported case. J Vasc Surg. 1997 Oct;26(4):704-10. doi: 10.1016/s0741-5214(97)70074-0. PMID: 9357476.

33. Hendriksz CJ, Lavery C, Coker M, Ucar SK, Jain M, Bell L, Lampe C. Burden of disease in patients with Morquio A syndrome: results from an international patient-reported outcomes survey. Orphanet J Rare Dis. 2014 Mar 7;9:32. doi: 10.1186/1750-1172-9-32. PMID: 24602160; PMCID: PMC4016149.

34. Sawamoto K, Karumuthil-Melethil S, Khan S, Stapleton M, Bruder JT, Danos O, Tomatsu S. Liver-Targeted AAV8 Gene Therapy Ameliorates Skeletal and Cardiovascular Pathology in a Mucopolysaccharidosis IVA Murine Model. Mol Ther Methods Clin Dev. 2020 May 22;18:50-61. doi: 10.1016/j.omtm.2020.05.015. PMID: 32577432; PMCID: PMC7301175.

35. Piechnik M, Amendum PC, Sawamoto K, Stapleton M, Khan S, Fnu N, Álvarez V, Pachon AMH, Danos O, Bruder JT, Karumuthil-Melethil S, Tomatsu S. Sex Difference Leads to Differential Gene Expression Patterns and Therapeutic Efficacy in Mucopolysaccharidosis IVA Murine Model Receiving AAV8 Gene Therapy. Int J Mol Sci. 2022 Oct 21;23(20):12693. doi: 10.3390/ijms232012693. PMID: 36293546; PMCID: PMC9604118.

36. Neufeld EF, Muenzer J. The Mucopolysaccharidoses. In: The Online Metabolic and Molecular Bases of Inherited Disease [Internet]. McGraw-Hill Education; 2019 [cited 2024]. Available from: https://ommbid.mhmedical.com/content.aspx?sectionid=225544161&bookid=2709

37. Orii T, Kiman T, and Sukegawa K. Late onset N-acetylgalactosamine-6-sulfate sulfatase deficiency in two brothers. 1981;13:169-75. ***Literature copy not provided.***

38. Sukegawa K, Orii T. Residual activity in fibroblasts from two brothers with the late-onset form of N-acetylgalactosamine-6-sulphate sulphatase deficiency. J Inherit Metab Dis. 1982;5(4):231-2. doi: 10.1007/BF02179150. PMID: 6820446.

39. Tomatsu S, Yasuda E, Patel P, Ruhnke K, Shimada T, Mackenzie WG, Mason R, Thacker MM, Theroux M, Montaño AM, Alméciga-Díaz CJ, Barrera LA, Chinen Y, Sly WS, Rowan D, Suzuki Y, Orii T. Morquio A syndrome: diagnosis and current and future therapies. Pediatr Endocrinol Rev. 2014 Sep;12 Suppl 1(0 1):141-51. PMID: 25345096; PMCID: PMC4259875.

40. Theroux MC, DiCindio S, Averill LW, Pizarro C, Oommen A, Bober MB, Ditro C, Campbell J, Duker AL, Jones T, Passi V, Barth P, Schmidt RJ, Little M, Mackenzie S, Tomatsu S, Mackenzie WG. Tracheal Narrowing and Its Impact on Anesthesia Care in Patients With Morquio A (Mucopolysaccharidosis Type IVA): An Observational Study. Anesth Analg. 2023 Nov 1;137(5):1075-1083. doi: 10.1213/ANE.0000000000006513. Epub 2023 May 9. PMID: 37862598; PMCID: PMC10634225.

41. Doherty C, Averill LW, Theroux M, Mackenzie WG, Pizarro C, Mason RW, Tomatsu S. Natural history of Morquio A patient with tracheal obstruction from birth to death. Mol Genet Metab Rep. 2017 Dec 22;14:59-67. doi: 10.1016/j.ymgmr.2017.11.005. PMID: 29326877; PMCID: PMC5758848.

42. Yasuda E, Fushimi K, Suzuki Y, Shimizu K, Takami T, Zustin J, Patel P, Ruhnke K, Shimada T, Boyce B, Kokas T, Barone C, Theroux M, Mackenzie W, Nagel B, Ryerse JS, Orii KE, Iida H, Orii T, Tomatsu S. Pathogenesis of Morquio A syndrome: an autopsied case reveals systemic storage disorder. Mol Genet Metab. 2013 Jul;109(3):301-11. doi: 10.1016/j.ymgme.2013.04.009. Epub 2013 Apr 16. PMID: 23683769.

43. Hendriksz CJ, Burton B, Fleming TR, Harmatz P, Hughes D, Jones SA, Lin SP, Mengel E, Scarpa M, Valayannopoulos V, Giugliani R; STRIVE Investigators; Slasor P, Lounsbury D, Dummer W. Efficacy and safety of enzyme replacement therapy with BMN 110 (elosulfase alfa) for Morquio A syndrome (mucopolysaccharidosis IVA): a phase 3 randomised placebo-controlled study. J Inherit Metab Dis. 2014 Nov;37(6):979-90. doi: 10.1007/s10545-014-9715-6. Epub 2014 May 9. PMID: 24810369; PMCID: PMC4206772.

44. Hendriksz CJ, Giugliani R, Harmatz P, Mengel E, Guffon N, Valayannopoulos V, Parini R, Hughes D, Pastores GM, Lau HA, Al-Sayed MD, Raiman J; STRIVE Investigators; Yang K, Mealiffe M, Haller C. Multi-domain impact of elosufase alfa in Morquio A syndrome in the pivotal phase III trial. Mol Genet Metab. 2015 Feb;114(2):178-85. doi: 10.1016/j.ymgme.2014.08.012. Epub 2014 Sep 6. PMID: 25284089.

45. Hendriksz CJ, Berger KI, Giugliani R, Harmatz P, Kampmann C, Mackenzie WG, Raiman J, Villarreal MS, Savarirayan R. International guidelines for the management and treatment of Morquio A syndrome. Am J Med Genet A. 2015 Jan;167A(1):11-25. doi: 10.1002/ajmg.a.36833. Epub 2014 Oct 24. PMID: 25346323; PMCID: PMC4309407.

46. Tomatsu S, Sawamoto K, Shimada T, Bober MB, Kubaski F, Yasuda E, Mason RW, Khan S, Alméciga-Díaz CJ, Barrera LA, Mackenzie WG, Orii T. Enzyme replacement therapy for treating mucopolysaccharidosis type IVA (Morquio A syndrome): effect and limitations. Expert Opin Orphan Drugs. 2015 Nov 1;3(11):1279-1290. doi: 10.1517/21678707.2015.1086640. Epub 2015 Oct 29. PMID: 26973801; PMCID: PMC4788508.

47. Do Cao J, Wiedemann A, Quinaux T, Battaglia-Hsu SF, Mainard L, Froissart R, Bonnemains C, Ragot S, Leheup B, Journeau P, Feillet F. 30 months follow-up of an early enzyme replacement therapy in a severe Morquio A patient: About one case. Mol Genet Metab Rep. 2016 Oct 10;9:42-45. doi: 10.1016/j.ymgmr.2016.10.001. PMID: 27761411; PMCID: PMC5065040.

48. Frigeni M, Rodriguez-Buritica DF, Saavedra H, Gunther KA, Hillman PR, Balaguru D, Northrup H. The youngest pair of siblings with Mucopolysaccharidosis type IVA to receive enzyme replacement therapy to date: A case report. Am J Med Genet A. 2021 Nov;185(11):3510-3516. doi: 10.1002/ajmg.a.62469. Epub 2021 Sep 2. PMID: 34472180.

49. Yasuda E, Suzuki Y, Shimada T, Sawamoto K, Mackenzie WG, Theroux MC, Pizarro C, Xie L, Miller F, Rahman T, Kecskemethy HH, Nagao K, Morlet T, Shaffer TH, Chinen Y, Yabe H, Tanaka A, Shintaku H, Orii KE, Orii KO, Mason RW, Montaño AM, Fukao T, Orii T, Tomatsu S. Activity of daily living for Morquio A syndrome. Mol Genet Metab. 2016 Jun;118(2):111-22. doi: 10.1016/j.ymgme.2016.04.005. Epub 2016 Apr 25. PMID: 27161890; PMCID: PMC5016714.

50. Khan SA, Mason RW, Giugliani R, Orii K, Fukao T, Suzuki Y, Yamaguchi S, Kobayashi H, Orii T, Tomatsu S. Glycosaminoglycans analysis in blood and urine of patients with mucopolysaccharidosis. Mol Genet Metab. 2018 Sep;125(1-2):44-52. doi: 10.1016/j.ymgme.2018.04.011. Epub 2018 May 17. PMID: 29779903; PMCID: PMC6175648.

51. Fujitsuka H, Sawamoto K, Peracha H, Mason RW, Mackenzie W, Kobayashi H, Yamaguchi S, Suzuki Y, Orii K, Orii T, Fukao T, Tomatsu S. Biomarkers in patients with mucopolysaccharidosis type II and IV. Mol Genet Metab Rep. 2019 Feb 5;19:100455. doi: 10.1016/j.ymgmr.2019.100455. PMID: 30775257; PMCID: PMC6365937.

52. Tomatsu S, Montaño AM, Ohashi A, Gutierrez MA, Oikawa H, Oguma T, Dung VC, Nishioka T, Orii T, Sly WS. Enzyme replacement therapy in a murine model of Morquio A syndrome. Hum Mol Genet. 2008 Mar 15;17(6):815-24. doi: 10.1093/hmg/ddm353. Epub 2007 Dec 3. PMID: 18056156.

53. Qi Y, Musson DG, Schweighardt B, Tompkins T, Jesaitis L, Shaywitz AJ, Yang K, O'Neill CA. Pharmacokinetic and pharmacodynamic evaluation of elosulfase alfa, an enzyme replacement therapy in patients with Morquio A syndrome. Clin Pharmacokinet. 2014 Dec;53(12):1137-47. doi: 10.1007/s40262-014-0173-y. PMID: 25234648; PMCID: PMC4243006.

54. Nakashima Y, Tomatsu S, Hori T, Fukuda S, Sukegawa K, Kondo N, Suzuki Y, Shimozawa N, Orii T. Mucopolysaccharidosis IV A: molecular cloning of the human N-acetylgalactosamine-6-sulfatase gene (GALNS) and analysis of the 5'-flanking region.

Genomics. 1994 Mar 1;20(1):99-104. doi: 10.1006/geno.1994.1132. PMID: 8020961. ***Literature copy not provided.***

55. Masue M, Sukegawa K, Orii T, Hashimoto T. N-acetylgalactosamine-6-sulfate sulfatase in human placenta: purification and characteristics. J Biochem. 1991 Dec;110(6):965-70. doi: 10.1093/oxfordjournals.jbchem.a123697. PMID: 1794986.

56. Zhao L, Yang Z, Zheng M, Shi L, Gu M, Liu G, Miao F, Chang Y, Huang F, Tang N. Recombinant adeno-associated virus 8 vector in gene therapy: Opportunities and challenges. Genes Dis. 2023 Mar 24;11(1):283-293. doi: 10.1016/j.gendis.2023.02.010. PMID: 37588223; PMCID: PMC10425794.

57. Harmatz PR, Mengel KE, Giugliani R, Valayannopoulos V, Lin SP, Parini R, Guffon N, Burton BK, Hendriksz CJ, Mitchell JJ, Martins AM, Jones SA, Guelbert N, Vellodi A, Wijburg FA, Yang K, Slasor P, Decker C. Longitudinal analysis of endurance and respiratory function from a natural history study of Morquio A syndrome. Mol Genet Metab. 2015 Feb;114(2):186-94. doi: 10.1016/j.ymgme.2014.10.015. Epub 2014 Nov 1. PMID: 25582974.

58. Regier DS, Tanpaiboon P. Role of elosulfase alfa in mucopolysaccharidosis IVA. Appl Clin Genet. 2016 Jun 14;9:67-74. doi: 10.2147/TACG.S69080. PMID: 27366102; PMCID: PMC4913537.

59. Harmatz PR, Mengel E, Geberhiwot T, Muschol N, Hendriksz CJ, Burton BK, Jameson E, Berger KI, Jester A, Treadwell M, Sisic Z, Decker C. Impact of elosulfase alfa in patients with morquio A syndrome who have limited ambulation: An open-label, phase 2 study. Am J Med Genet A. 2017 Feb;173(2):375-383. doi: 10.1002/ajmg.a.38014. Epub 2016 Oct 24. PMID: 27774754; PMCID: PMC5298029.

60. Yabe H, Tanaka A, Chinen Y, Kato S, Sawamoto K, Yasuda E, Shintaku H, Suzuki Y, Orii T, Tomatsu S. Hematopoietic stem cell transplantation for Morquio A syndrome. Mol Genet Metab. 2016 Feb;117(2):84-94. doi: 10.1016/j.ymgme.2015.09.011. Epub 2015 Oct 1. PMID: 26452513; PMCID: PMC5016080.

61. Chinen Y, Higa T, Tomatsu S, Suzuki Y, Orii T, Hyakuna N. Long-term therapeutic efficacy of allogenic bone marrow transplantation in a patient with mucopolysaccharidosis IVA. Mol Genet Metab Rep. 2014;1:31-41. doi: 10.1016/j.ymgmr.2013.11.002. PMID: 25593792; PMCID: PMC4292891.

62. Chen H, Khan S, Celik B, Suzuki Y, Ago Y, Tomatsu S. Activity of daily living in mucopolysaccharidosis IVA patients: Evaluation of therapeutic efficacy. Mol Genet Genomic Med. 2021 Nov;9(11):e1806. doi: 10.1002/mgg3.1806. Epub 2021 Oct 8. PMID: 34623762; PMCID: PMC8606213.

63. Shimada T, Tomatsu S, Mason RW, Yasuda E, Mackenzie WG, Hossain J, Shibata Y, Montaño AM, Kubaski F, Giugliani R, Yamaguchi S, Suzuki Y, Orii KE, Fukao T, Orii T. Di-sulfated Keratan Sulfate as a Novel Biomarker for Mucopolysaccharidosis II, IVA, and IVB. JIMD Rep. 2015;21:1-13. doi: 10.1007/8904_2014_330. Epub 2015 Feb 25. PMID: 25712379; PMCID: PMC4470954.

64. Shimada T, Tomatsu S, Yasuda E, Mason RW, Mackenzie WG, Shibata Y, Kubaski F, Giugliani R, Yamaguchi S, Suzuki Y, Orii K, Orii T. Chondroitin 6-Sulfate as a Novel

Biomarker for Mucopolysaccharidosis IVA and VII. JIMD Rep. 2014;16:15-24. doi: 10.1007/8904_2014_311. Epub 2014 May 22. PMID: 24850234; PMCID: PMC4221613.

65. Tomatsu S, Montaño AM, Oguma T, Dung VC, Oikawa H, de Carvalho TG, Gutiérrez ML, Yamaguchi S, Suzuki Y, Fukushi M, Kida K, Kubota M, Barrera L, Orii T. Validation of keratan sulfate level in mucopolysaccharidosis type IVA by liquid chromatography-tandem mass spectrometry. J Inherit Metab Dis. 2010 Dec;33 Suppl 3:S35-42. doi: 10.1007/s10545-009-9013-x. Epub 2010 Jan 27. PMID: 20107903.

66. Tomatsu S, Orii KO, Vogler C, Nakayama J, Levy B, Grubb JH, Gutierrez MA, Shim S, Yamaguchi S, Nishioka T, Montano AM, Noguchi A, Orii T, Kondo N, Sly WS. Mouse model of N-acetylgalactosamine-6-sulfate sulfatase deficiency (Galns-/-) produced by targeted disruption of the gene defective in Morquio A disease. Hum Mol Genet. 2003 Dec 15;12(24):3349-58. doi: 10.1093/hmg/ddg366. Epub 2003 Oct 28. PMID: 14583446.

67. Tomatsu S, Gutierrez M, Nishioka T, Yamada M, Yamada M, Tosaka Y, Grubb JH, Montaño AM, Vieira MB, Trandafirescu GG, Peña OM, Yamaguchi S, Orii KO, Orii T, Noguchi A, Laybauer L. Development of MPS IVA mouse (Galnstm(hC79S.mC76S)slu) tolerant to human N-acetylgalactosamine-6-sulfate sulfatase. Hum Mol Genet. 2005 Nov 15;14(22):3321-35. doi: 10.1093/hmg/ddi364. Epub 2005 Oct 11. PMID: 16219627.

68. Tomatsu S, Vogler C, Montaño AM, Gutierrez M, Oikawa H, Dung VC, Orii T, Noguchi A, Sly WS. Murine model (Galns(tm(C76S)slu)) of MPS IVA with missense mutation at the active site cysteine conserved among sulfatase proteins. Mol Genet Metab. 2007 Jul;91(3):251-8. doi: 10.1016/j.ymgme.2007.02.009. Epub 2007 May 10. PMID: 17498992.

69. FDA Center for Drug Evaluation and Research [Internet]. 2013 [cited 2024 Mar]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/125554Orig1s000TOC.cfm

70. Ferla R, Alliegro M, Marteau JB, Dell'Anno M, Nusco E, Pouillot S, Galimberti S, Valsecchi MG, Zuliani V, Auricchio A. Non-clinical Safety and Efficacy of an AAV2/8 Vector Administered Intravenously for Treatment of Mucopolysaccharidosis Type VI. Mol Ther Methods Clin Dev. 2017 Jul 24;6:143-158. doi: 10.1016/j.omtm.2017.07.004. PMID: 28932756; PMCID: PMC5552066.

71. Wang L, Warzecha CC, Kistner A, Chichester JA, Bell P, Buza EL, He Z, Pampena MB, Couthouis J, Sethi S, McKeever K, Betts MR, Kakkis E, Wilson JM, Wadsworth S, Sullivan BA. Prednisolone reduces the interferon response to AAV in cynomolgus macaques and may increase liver gene expression. Mol Ther Methods Clin Dev. 2022 Jan 19;24:292-305. doi: 10.1016/j.omtm.2022.01.007. PMID: 35211641; PMCID: PMC8841522.

72. Childers MK, Joubert R, Poulard K, Moal C, Grange RW, Doering JA, Lawlor MW, Rider BE, Jamet T, Danièle N, Martin S, Rivière C, Soker T, Hammer C, Van Wittenberghe L, Lockard M, Guan X, Goddard M, Mitchell E, Barber J, Williams JK, Mack DL, Furth ME, Vignaud A, Masurier C, Mavilio F, Moullier P, Beggs AH, Buj-Bello A. Gene therapy prolongs survival and restores function in murine and canine models of myotubular myopathy. Sci Transl Med. 2014 Jan 22;6(220):220ra10. doi: 10.1126/scitranslmed.3007523. PMID: 24452262; PMCID: PMC4105197.

73. Greig JA, Limberis MP, Bell P, Chen SJ, Calcedo R, Rader DJ, Wilson JM. Non-Clinical Study Examining AAV8.TBG.hLDLR Vector-Associated Toxicity in Chow-Fed Wild-Type and

LDLR+/- Rhesus Macaques. Hum Gene Ther Clin Dev. 2017 Mar;28(1):39-50. doi: 10.1089/humc.2017.014. PMID: 28319449; PMCID: PMC5369385.

74. Mendell JR, Al-Zaidy SA, Rodino-Klapac LR, Goodspeed K, Gray SJ, Kay CN, Boye SL, Boye SE, George LA, Salabarria S, Corti M, Byrne BJ, Tremblay JP. Current Clinical Applications of In Vivo Gene Therapy with AAVs. Mol Ther. 2021 Feb 3;29(2):464-488. doi: 10.1016/j.ymthe.2020.12.007. Epub 2020 Dec 10. PMID: 33309881; PMCID: PMC7854298.

75. Nathwani AC, Reiss UM, Tuddenham EG, Rosales C, Chowdary P, McIntosh J, Della Peruta M, Lheriteau E, Patel N, Raj D, Riddell A, Pie J, Rangarajan S, Bevan D, Recht M, Shen YM, Halka KG, Basner-Tschakarjan E, Mingozzi F, High KA, Allay J, Kay MA, Ng CY, Zhou J, Cancio M, Morton CL, Gray JT, Srivastava D, Nienhuis AW, Davidoff AM. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. N Engl J Med. 2014 Nov 20;371(21):1994-2004. doi: 10.1056/NEJMoa1407309. PMID: 25409372; PMCID: PMC4278802.

76. Dowling JJ, Müller-Felber W, Smith BK, Bönnemann CG, Kuntz NL, Muntoni F, Servais L, Alfano LN, Beggs AH, Bilder DA, Blaschek A, Duong T, Graham RJ, Jain M, Lawlor MW, Lee J, Coats J, Lilien C, Lowes LP, MacBean V, Neuhaus S, Noursalehi M, Pitts T, Finlay C, Christensen S, Rafferty G, Seferian AM, Tsuchiya E, James ES, Miller W, Sepulveda B, Vila MC, Prasad S, Rico S, Shieh PB; INCEPTUS investigators. INCEPTUS Natural History, Run-in Study for Gene Replacement Clinical Trial in X-Linked Myotubular Myopathy. J Neuromuscul Dis. 2022;9(4):503-516. doi: 10.3233/JND-210781. PMID: 35694931; PMCID: PMC9398079.

77. NIH US National Library of Medicine. Gene Therapy in Patients with Mucopolysaccharidosis Disease [Internet]. 2017 [cited 2024 Mar]. Available from: https://clinicaltrials.gov/study/NCT03173521

78. Song S, Scott-Jorgensen M, Wang J, Poirier A, Crawford J, Campbell-Thompson M, Flotte TR. Intramuscular administration of recombinant adeno-associated virus 2 alpha-1 antitrypsin (rAAV-SERPINA1) vectors in a nonhuman primate model: safety and immunologic aspects. Mol Ther. 2002 Sep;6(3):329-35. doi: 10.1006/mthe.2002.0673. PMID: 12231168.

79. Tylki-Szymańska A, Almássy Z, Christophidou-Anastasiadou V, Avdjieva-Tzavella D, Barisic I, Cerkauskiene R, Cuturilo G, Djiordjevic M, Gucev Z, Hlavata A, Kieć-Wilk B, Magner M, Pecin I, Plaiasu V, Samardzic M, Zafeiriou D, Zaganas I, Lampe C. The landscape of Mucopolysaccharidosis in Southern and Eastern European countries: a survey from 19 specialistic centers. Orphanet J Rare Dis. 2022 Mar 24;17(1):136. doi: 10.1186/s13023-022-02285-x. PMID: 35331284; PMCID: PMC8943501.

80. Çelik B, Tomatsu SC, Tomatsu S, Khan SA. Epidemiology of Mucopolysaccharidoses Update. Diagnostics (Basel). 2021 Feb 10;11(2):273. doi: 10.3390/diagnostics11020273. PMID: 33578874; PMCID: PMC7916572.

81. Applegarth DA, Toone JR, Lowry RB. Incidence of inborn errors of metabolism in British Columbia, 1969-1996. Pediatrics. 2000 Jan;105(1):e10. doi: 10.1542/peds.105.1.e10. PMID: 10617747.

82. Lowry RB, Applegarth DA, Toone JR, MacDonald E, Thunem NY. An update on the frequency of mucopolysaccharide syndromes in British Columbia. Hum Genet. 1990 Aug;85(3):389-90. doi: 10.1007/BF00206770. PMID: 2118475.

83. Nelson J, Crowhurst J, Carey B, Greed L. Incidence of the mucopolysaccharidoses in Western Australia. Am J Med Genet A. 2003 Dec 15;123A(3):310-3. doi: 10.1002/ajmg.a.20314. PMID: 14608657.

84. Pinto R, Caseiro C, Lemos M, Lopes L, Fontes A, Ribeiro H, Pinto E, Silva E, Rocha S, Marcão A, Ribeiro I, Lacerda L, Ribeiro G, Amaral O, Sá Miranda MC. Prevalence of lysosomal storage diseases in Portugal. Eur J Hum Genet. 2004 Feb;12(2):87-92. doi: 10.1038/sj.ejhg.5201044. PMID: 14685153.

85. Baehner F, Schmiedeskamp C, Krummenauer F, Miebach E, Bajbouj M, Whybra C, Kohlschütter A, Kampmann C, Beck M. Cumulative incidence rates of the mucopolysaccharidoses in Germany. J Inherit Metab Dis. 2005;28(6):1011-7. doi: 10.1007/s10545-005-0112-z. PMID: 16435194.

86. Poorthuis BJ, Wevers RA, Kleijer WJ, Groener JE, de Jong JG, van Weely S, Niezen-Koning KE, van Diggelen OP. The frequency of lysosomal storage diseases in The Netherlands. Hum Genet. 1999 Jul-Aug;105(1-2):151-6. doi: 10.1007/s004399900075. PMID: 10480370.

87. Poupetová H, Ledvinová J, Berná L, Dvoráková L, Kozich V, Elleder M. The birth prevalence of lysosomal storage disorders in the Czech Republic: comparison with data in different populations. J Inherit Metab Dis. 2010 Aug;33(4):387-96. doi: 10.1007/s10545-010-9093-7. Epub 2010 May 20. PMID: 20490927; PMCID: PMC2903693.

88. Malm G, Lund AM, Månsson JE, Heiberg A. Mucopolysaccharidoses in the Scandinavian countries: incidence and prevalence. Acta Paediatr. 2008 Nov;97(11):1577-81. doi: 10.1111/j.1651-2227.2008.00965.x. Epub 2008 Aug 4. Erratum in: Acta Paediatr. 2008 Dec;97(12):1773. PMID: 18681890.

89. Nelson J. Incidence of the mucopolysaccharidoses in Northern Ireland. Hum Genet. 1997 Dec;101(3):355-8. doi: 10.1007/s004390050641. PMID: 9439667.

90. Jurecka A, Ługowska A, Golda A, Czartoryska B, Tylki-Szymańska A. Prevalence rates of mucopolysaccharidoses in Poland. J Appl Genet. 2015 May;56(2):205-10. doi: 10.1007/s13353-014-0262-5. Epub 2014 Dec 4. PMID: 25472774; PMCID: PMC4412687.

91. Khan SA, Peracha H, Ballhausen D, Wiesbauer A, Rohrbach M, Gautschi M, Mason RW, Giugliani R, Suzuki Y, Orii KE, Orii T, Tomatsu S. Epidemiology of mucopolysaccharidoses. Mol Genet Metab. 2017 Jul;121(3):227-240. doi: 10.1016/j.ymgme.2017.05.016. Epub 2017 May 26. PMID: 28595941; PMCID: PMC5653283.

92. Meikle PJ, Ranieri E, Ravenscroft EM, Hua CT, Brooks DA, Hopwood JJ. Newborn screening for lysosomal storage disorders. Southeast Asian J Trop Med Public Health. 1999;30 Suppl 2:104-10. PMID: 11400743.

93. Josahkian JA, Trapp FB, Burin MG, Michelin-Tirelli K, Magalhães APPS, Sebastião FM, Bender F, Mari JF, Brusius-Facchin AC, Leistner-Segal S, Málaga DR, Giugliani R. Updated birth prevalence and relative frequency of mucopolysaccharidoses across Brazilian regions. Genet Mol Biol. 2021 Jan 27;44(1):e20200138. doi: 10.1590/1678-4685-GMB-2020-0138. PMID: 33503199; PMCID: PMC7839630.

94. Gómez AM, García-Robles R, Suárez-Obando F. Estimación de las frecuencias de las mucopolisacaridosis y análisis de agrupamiento espacial en los departamentos de Cundinamarca y Boyacá [Estimation of the mucopolysaccharidoses frequencies and cluster

analysis in the Colombian provinces of Cundinamarca and Boyacá]. Biomedica. 2012 Oct-Dec;32(4):602-9. Spanish. doi: 10.1590/S0120-41572012000400015. PMID: 23715235.

95. Mendoza-Ruvalcaba SDC, Brambila-Tapia AJL, Juárez-Osuna JA, Silva-José TDD, García-Ortiz JE. Biochemical diagnosis of mucopolysaccharidosis in a Mexican reference center. Genet Mol Biol. 2020 Feb 14;43(1):e20180347. doi: 10.1590/1678-4685-GMB-2018-0347. PMID: 32106281; PMCID: PMC7198031.

96. Lin HY, Lin SP, Chuang CK, Niu DM, Chen MR, Tsai FJ, Chao MC, Chiu PC, Lin SJ, Tsai LP, Hwu WL, Lin JL. Incidence of the mucopolysaccharidoses in Taiwan, 1984-2004. Am J Med Genet A. 2009 May;149A(5):960-4. doi: 10.1002/ajmg.a.32781. PMID: 19396827.

97. Moammar H, Cheriyan G, Mathew R, Al-Sannaa N. Incidence and patterns of inborn errors of metabolism in the Eastern Province of Saudi Arabia, 1983-2008. Ann Saudi Med. 2010 Jul-Aug;30(4):271-7. doi: 10.4103/0256-4947.65254. PMID: 20622343; PMCID: PMC2931777.

98. Cho SY, Sohn YB, Jin DK. An overview of Korean patients with mucopolysaccharidosis and collaboration through the Asia Pacific MPS Network. Intractable Rare Dis Res. 2014 Aug;3(3):79-86. doi: 10.5582/irdr.2014.01013. PMID: 25364648; PMCID: PMC4214241.

99. Nasrallah F, Oueslati MAA, Hadj-Taieb S, Qurashi NA, Aboalmagd HI, Zidi W, Ferchichi S, Feki M, Sanhaji H. Incidence of the Mucopolysaccharidoses in Tunisia, 1999 - 2021. Clin Lab. 2023 Nov 1;69(11). doi: 10.7754/Clin.Lab.2023.230330. PMID: 37948494.

100. Michelakakis H, Dimitriou E, Tsagaraki S, Giouroukos S, Schulpis K, Bartsocas CS. Lysosomal storage diseases in Greece. Genet Couns. 1995;6(1):43-7. PMID: 7794561.

101. Terzioglu M, Tokatli A, Coskun T, Emre S. Molecular analysis of Turkish mucopolysaccharidosis IVA (Morquio A) patients: identification of novel mutations in the N-acetylgalactosamine-6-sulfate sulfatase (GALNS) gene. Hum Mutat. 2002 Dec;20(6):477-8. doi: 10.1002/humu.9088. PMID: 12442278.

102. Tylki-Szymańska A, Almássy Z, Christophidou-Anastasiadou V, Avdjieva-Tzavella D, Barisic I, Cerkauskiene R, Cuturilo G, Djiordjevic M, Gucev Z, Hlavata A, Kieć-Wilk B, Magner M, Pecin I, Plaiasu V, Samardzic M, Zafeiriou D, Zaganas I, Lampe C. The landscape of Mucopolysaccharidosis in Southern and Eastern European countries: a survey from 19 specialistic centers. Orphanet J Rare Dis. 2022 Mar 24;17(1):136. doi: 10.1186/s13023-022-02285-x. PMID: 35331284; PMCID: PMC8943501.

11 APPENDIX 1

Human GALNS (hGALNS)	atggcggcggttgtcgcggcgacgaggtggtggcagctgttgctggtgctcagcgc cgcggggatgggggcctcgggcgccccgcagcccccaacatcctgctcctgctc atggacgacatgggatggggtgacctcggggtgtatggagagccctccagagaga ccccgaatttggaccggatggctgcagaagggctgcttttcccaaacttctattctgcc aaccctctgtgctcgccatcgagggcggcactgctcacaggacggctacccatccg caatggcttctacaccaccaacgcccatgccagaaacgcctacacaccgcaggaga ttgtgggcggcatcccagactcggagcagctcctgccggagcttctgaagaaggcc ggctacgtcagcaagattgtcggcaagtggcatctgggtcacaggccccagttccac cccctgaagcacggatttgatgagtggtttggatccccccaactgccactttggaccttа tgacaacaaggccaggcccaacatccctgtgtacagggactgggagatggttggca gatattatgaagaatttcctattaatctgaagacgggggaagccaacctcacccagat ctacctgcaggaagccctggacttcattaagagacaggcacggcaccacccctttttc ctctactgggctgtcgacgccacgcacgcacccgtctatgcctccaaacccttcttgg gcaccagtcagcgagggcggtatggagacgccgtccgggagattgatgacagcat tgggaagatactggagctcctccaagacctgcacgtcgcggacaacaccttcgtctt cttcacgtcggacaacggcgctgccctcatttccgcccccgaacaaggtggcagca acggcccctttctgtgtgggaagcagaccacgtttgaaggagggatgagggagcct gccctcgcatggtggccagggcacgtcactgcaggccaggtgagccaccagctgg gcagcatcatggacctcttcaccaccagcctggcccttgcgggcctgacgccgccc agcgacagggccattgatggcctcaacctcctccccaccctcctgcagggccggct gatggacaggcctatcttctattaccgtggcgacacgctgatggcggccaccctcgg gcagcacaaggctcacttctggacctggaccaactcctgggagaacttcagacagg gcattgatttctgccctgggcagaacgtttcaggggtcacaactcacaatctggaaga ccacacgaagctgcccctgatcttccacctgggacgggacccaggggagaggttcc ccctcagctttgccagcgccgagtaccaggaggccctcagcaggatcacctcggtc gtccagcagcaccaggaggccttggtccccgcgcagccccagctcaacgtgtgca actgggcggtcatgaactgggcacctccgggctgtgaaaagttagggaagtgtctg acacctccagaatccattcccaagaagtgcctctggtcccactag

12 APPENDIX 2

Tabulated Summary of Epidemiological Analyses of MPS IVA [References: 3,13,80,83-84,89,93,95,102]

Country	Prevalence of MPS IVA
British Columbia	0.48 per 100,000 births
Northern Ireland	0.86 per 100,000 births
Australia	0.59 per 100,000 births
Portugal	0.6 per 100,000 births
Western Australia	0.15 per 100,000 births
Brazil	0.15 per 100,000 births
Mexico	1.10 per 100,000 births
Japan	0.15 per 100,000 births
United States	0.03 per 100,000 births
Czech Republic	0.71 per 100,000 births
Denmark*	0.31 per 100,000 births
Germany	0.38 per 100,000 births
Norway*	0.24 per 100,000 births
Poland*	0.14 per 100,000 births
Sweden*	0.07 per 100,000 births
Switzerland*	0.38 per 100,000 births
The Netherlands	0.22 per 100,000 births
Columbia*	0.68 per 100,000 births
Taiwan	0.33 per 100,000 births
Saudi Arabia*	3.6 per 100,000 births
South Korea*	0.13 per 100,000 births
Tunisia*	0.68 per 100,000 births

*Represent all MPS IV cases (A and B).

Country	Commonality of MPS IVA
India	Third most common MPS
United States	Third most common MPS
Most of Europe	Third most common MPS
Southern Europe	Second most common MPS, behind MPS II
Eastern Europe	Second most common MPS, behind MPS II

MPS IVA is the third most common MPS in India, the United States, and most of Europe and the second most common MPS behind MPS II in Southern and Eastern Europe [3].

1. **Studies performed in the United States**
    a. **Current MPS IVA Natural History Program at NCH-DE**

Nemours Children's Health in Wilmington, DE is a well-known hub for Morquio A patients in the United States and globally, given the plethora of Morquio experts in orthopedics, cardiovascular, respiratory and pulmonology, and anesthesiology. NCH-DE currently has 178 MPS IVA patients registered in the system indicated as having a Morquio A Syndrome diagnosis. In the research study, key clinical features are assessed non-invasively on MPS IVA patients, including various questionnaires (Quality of Life, Activities of Daily Living, Medical History, etc.), pulmonary function, skeletal imaging, bone mineralization, gait pattern, joint laxity, tracheal function, and hearing function. Patients have a total of four visits for research evaluations occurring every 18 months. There are currently 60 participants enrolled in the Natural History Program. 53% are female and 47% are male. 43% are 0-10 years old; 18% are 11-20 years old; 22% are 21-30 years old; 7% are 31-40 years old; and 10% are 41 years or older. Approximately 5% of the participants are Black or African American, 5% are Asian, 73% are White or Caucasian, and 17% are other/multiple races. 52% of participants reside in the South of the United States, 18% in the Northeast, 10% in the Midwest, 12% in the West, and 8% internationally. 57 participants completed their baseline visits, and 27 participants completed their second visits. 15 surgical samples have been collected from patients. Study data analysis is ongoing (Data is current as of 03/27/2024).

b. **MPS Incidence in the United States**

MPS Type	**Incidence Rate in the US**
MPS I	0.26 per 100,000 births
MPS II	0.26 per 100,000 births
MPS III	0.26 per 100,000 births
MPS IV	0.14 per 100,000 births
MPS VI	0.04 per 100,000 births
MPS VII	0.027 per 100,000 births
MPS IX	No cases reported

MPS I, II, and III have the greatest incidence in the US, with 0.26 per 100,000 live births, followed by MPS IV at 0.14, MPS VI at 0.04, and MPS VII at 0.027 [3]. MPS IX has yet to have any reported cases in the United States [3]. This population study is based on the registry of the National MPS Society; therefore, the incidence is underestimated.

Appendix 2: Studies performed in countries of the EU

Reference	Poupětová, et al. 2010
Type of Source	Literature (peer-reviewed journal)
Region	*Czech Republic*
Collection Year(s)	1975-2008
Case Definition	MPS IVA
Data collection method	The prevalence was calculated as the total number of patients diagnosed with MPS IVA, divided by the total number of live births in the same period. Birth prevalence was expressed as the number of patients per 100,000 live births.
Design	Retrospective epidemiological survey
Reference population size	Live births 1975-2008: 4,261,897 (Czech Statistical Office web page)
Total number of patients with MPS IVA	15 MPS IV Type A 14 Type B 1
Prevalence (number per 100,000 live births)	0.73
Comments	• The initial diagnosis was based on demonstration of accumulated substrates (glycosaminoglycans) in tissues and/or body fluids using chromatographic or electrophoretic methods, or on histological proof of storage. • The definite diagnosis was made by demonstrating the deficiency of the relevant enzyme and/or presence of pathogenic mutation, and/or by detection of undegraded substrate by loading tests in cell cultures.

Reference	Malm, et al. 2008
Type of Source	Literature (peer-reviewed journal)
Region	*Denmark*
Collection Year(s)	1975-2004
Case Definition	MPS IV
Data collection method	The retrospective period used for the incidence study covered the period from 1975 to 2004. Prevalence was derived from the number of MPS patients alive as of December 31, 2007.
Design	Retrospective epidemiological survey
Reference population size	Live births 1975-2004: 3,100,000
Total number of patients with MPS IV	9 MPS IV Type A not specified Type B not specified
Prevalence (number per 100,000 live births)	0.48
Comments	• Selective screening for MPS was performed by analysis of GAGs in urine (heparan-, dermatan- and keratansulphate) in persons with a clinically suspected MPS. • When urinary levels of GAGs are increased, the findings of a low or deficient enzyme level in lymphocytes or fibroblasts confirm the diagnosis of MPS.

Reference	Michelakakis et al. 1995
Type of Source	Literature
Region	*Greece*
Collection Year(s)	Last 13 years
Case Definition	MPS IVA
Data collection method	Relative frequency of MPS IVA expressed as % of total diagnosed cases with lysosomal storage disease
Design	Survey
Reference population size	36 patients with lysosomal storage disease
Number of patients with MPS IVA	1
Prevalence (number per 100,000 live births)	Not calculated
Comments	• Diagnosis confirmed by quantification of GAGs quantified, enzyme assays • Data not related to demographic data • % of patients with LSD: 2.7

Reference	Baehner et al. 2005
Type of Source	Literature (peer-reviewed journal)
Region	*Germany*
Collection Year(s)	1980-1995
Case Definition	MPS IV
Data collection method	Crude occurrence rates (total number of cases divided by total number of births during study period)
Design	Retrospective epidemiological survey
Reference population size	Live births 1980-1995: 13,410,924 (German Bureau of Statistics)
Total number of patients with MPS IVA	52 MPS IV Type A 51 Type B 2
Cumulative Prevalence (number per 100,000 live births)	0.38
Comments	• In all cases diagnosis confirmed by enzyme assay in serum, leukocytes and/or fibroblasts • Prenatal diagnoses not included • Large proportion of Turkish population (11/51 MPS IVA patients of Turkish origin) • CIR for MPSs: 3.53 in 100,000 live births

Reference	Nelson 1997
Type of Source	Literature (peer-reviewed journal)
Region	*Northern Ireland*
Collection Year(s)	1958-1985
Case Definition	MPS IVA
Data collection method	Prenatal diagnosis included
Design	Survey
Reference population size	Live births 1958-1985: 839,517 (The Registrar General, Northern Ireland)
Number of patients with MPS IVA	11
Prevalence (number per 100,000 live births)	1.31
Comments	• Diagnosis confirmed by enzyme assay • Marked heterogeneity in the clinical presentation of the patients • Overall incidence of MPSs: 1 in 25,000 live births

Reference	Malm, et al. 2008
Type of Source	Literature (peer-reviewed journal)
Region	*Norway*
Collection Year(s)	1979-2004
Case Definition	MPS IV
Data collection method	The retrospective period used for the incidence study covered the period from 1979 to 2004. Prevalence was derived from the number of MPS patients alive as of December 31, 2007.
Design	Retrospective epidemiological survey
Reference population size	Live births 1979-2004: 2420000
Total number of patients with MPS IV	11 MPS IV Type A not specified Type B not specified
Prevalence (number per 100,000 live births)	0.76
Comments	• Most of the patients were from Pakistani origin. • Selective screening for MPS was performed by analysis of GAGs in urine (heparan-, dermatan- and keratansulphate) in persons with a clinically suspected MPS. • When urinary levels of GAGs are increased, the findings of a low or deficient enzyme level in lymphocytes or fibroblasts confirm the diagnosis of MPS.

Reference	Jurecka et al. 2015
Type of Source	Literature (peer-reviewed journal)
Region	*Poland*
Collection Year(s)	1970-2010
Case Definition	MPS IVA
Data collection method	The initial diagnosis of was based on the demonstration of accumulated substrates (GAGs) in body fluids using electrophoretic methods. The definite diagnosis was made by demonstrating the deficiency of the relevant enzyme and/or presence of pathogenic mutation.
Design	A retrospective epidemiological survey
Reference population size	Live births 1970-2010: 21,686,890
Number of patients with MPS IV	31
Prevalence (number per 100,000 live births)	0.14
Comments	• Diagnoses by accumulated GAGs. determination of enzyme activities • The confirmation of diagnosis was made by deficiency of the enzyme.

Reference	Pinto et al. 2004
Type of Source	Literature (peer-reviewed journal)
Region	*Portugal*
Collection Year(s)	1982-2001 (Portugal) 1979-1998 (Northern Portugal)
Case Definition	MPS IVA
Data collection method	Total number of diagnosed cases (post- and prenatal diagnosis) divided by total number of live births
Design	Survey
Reference population size	Number of live births not cited Provided by Instituto Nacional de Estatistica, Porto, Portugal
Number of patients with MPS IVA	11 (pre- and postnatal, 10 postnatal) 6 Northern Portugal (1979-1998)
Prevalence (number per 100,000 live births)	Not calculated
Comments	• Diagnoses by determination of enzyme activities • Pre- and postnatal diagnoses included • Birth incidence calculated using the more extensively studied Northern Portuguese population data • Overall birth incidence of MPSs: 4,8 per 100,000 live births (Northern Portugal)

Reference	Malm et al. 2008
Type of Source	Literature (peer-reviewed journal)
Region	*Sweden*
Collection Year(s)	1975-2004 1990-2009 Malin et al. 2014
Case Definition	MPS IVA
Data collection method	The retrospective period used for the incidence study covered the period from 1975 to 2004. Prevalence was derived from the number of MPS patients alive as of December 31, 2007.
Design	Retrospective epidemiological survey
Reference population size	Live births 1975-2004: 760,000
Total number of patients with MPS IVA	2 MPS IVA 6 MPS IVA (Malin et al. 2014)
Prevalence (number per 100,000 live births)	0.07 0.29 (Malin et al. 2014)
Comments	Seven persons with MPS IV lived in Sweden 2007; four of them (two pair of siblings) were immigrants (Malm et al. 2008).

Reference	Khan et al. 2017
Type of Source	Literature (peer-reviewed journal)
Region	*Switzerland*
Collection Year(s)	1975-2008
Case Definition	MPS IVA
Data collection method	Diagnosed either by measurement of reduced enzyme activity in leukocytes or fibroblasts and/or by molecular analysis.
Design	Epidemiology
Reference population size	Live births 1975-2008: 2,621,036
Total number of patients with MPS IVA	10
Prevalence (number per 100,000 live births)	0.38
Comments	• Abnormal urinary quantitative or qualitative GAGs alone 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.

Reference	Poorthuis et al. 1999
Type of Source	Literature (peer-reviewed journal)
Region	*The Netherlands*
Collection Year(s)	1970-1996
Case Definition	MPS IVA
Data collection method	Birth Incidence: total number of MPS IVA within a certain period divided by the total number of live births within the same period
Design	Survey
Reference population size	Live births 1951-1996: 9,639,257 (Dutch Central Bureau of Statistics)
Number of patients with MPS IVA	22 (1970-1996) 21 (Years of birth 1951-1996)
Prevalence (number per 100,000 live births)	0.36
Comments	• Pre- and postnatal diagnosis included • Birth incidence of MPSs: 4.5 per 100,000 live births

Appendix 3: Studies performed in other countries

Reference	Meikle et al. 1999
Type of Source	Literature (peer-reviewed journal)
Region	*Australia*
Collection Year(s)	1980-1996
Case Definition	MPS IVA
Data collection method	Number of postnatal plus prenatal diagnoses divided by number of births during study period
Design	Retrospective case study
Reference population size	Live births 1980-1996: 4,222,323 (Australian Bureau of Statistics, Canberra)
Number of patients with MPS IVA	25 (21 postnatal, 4 prenatal)
Prevalence (number per 100,000 live births)	Not calculated
Comments	• Enzymatic diagnosis • Pre- and postnatal diagnosis included • Incidence of MPS IVA: 1 per 201,000 births

Reference	Nelson et al. 2003
Type of Source	Literature (peer-reviewed journal)
Region	Western Australia
Collection Year(s)	1969-1996
Case Definition	MPS IVA
Data collection method	Total number of cases diagnosed pre- and postnatally divided by total number of births during the study period
Design	Epidemiological study
Reference population size	Live births 1969-1996: 641,178 (Australian Bureau of Statistics)
Number of patients with MPS IVA	1 (0 diagnosed prenatally)
Prevalence (number per 100,000 live births)	0.15
Comments	Diagnosis confirmed by one-dimensional electrophoresis of urinary GAGs and/or by enzyme assay on leucocytes or fibroblasts

Reference	Chen et al. 2016
Type of Source	Literature (peer-reviewed journal)
Region	*China*
Collection Year(s)	2006-2012
Case Definition	MPS IVA
Data collection method	Patients clinically suspected of having LSD based on physical symptoms were tested for the respective LSDs
Design	Survey
Reference population size	Total births 2006-2012: Not reported
Number of patients with MPS IVA	51
Prevalence (number per 100,000 live births)	Not reported
Comments	Various LSDs including MPA IV were diagnosed by enzymatic assays in Eastern China.

Reference	Sheth et al. 2013
Type of Source	Literature (peer-reviewed journal)
Region	*India*
Collection Year(s)	2002-2012
Case Definition	MPS IVA
Data collection method	Urine and enzyme assay
Design	Randomized study
Reference population size	Total births 2002-2012: Not reported
Number of patients with MPS IVA	18
Prevalence (number per 100,000 live births)	Not reported
Comments	Diagnosis was confirmed by enzymatic assays.

Reference	Khan et al. 2017
Type of Source	Literature (peer-reviewed journal)
Region	*Japan*
Collection Year(s)	1982-2009
Case Definition	MPS IVA
Data collection method	Screening of urinary GAGs
Design	Epidemiology
Reference population size	Not reported
Number of patients with MPS IVA	30
Prevalence (number per 100,000 live births)	Not calculated
Comments	Uronic acid quantitative test was carried out using an acid carbazole.

Reference	Omar et al. 2019
Type of Source	Literature (peer-reviewed journal)
Region	*Malaysia*
Collection Year(s)	2014-2016
Case Definition	MPS IVA
Data collection method	Screening high risk patients
Design	Screening
Reference population size	Not reported
Number of patients with MPS IVA	1
Prevalence (number per 100,000 live births)	Not reported
Comments	• Urine and whole blood samples were taken from high-risk MPS patients. • All urine samples were analysed for GAGs and characterised by high resolution electrophoresis

Reference	Cheema et al. 2017
Type of Source	Literature (peer-reviewed journal)
Region	*Pakistan*
Collection Year(s)	2013-2015
Case Definition	MPS IVA
Data collection method	Descriptive observational study Department of Pediatric Gastroenterology, Hepatology and Nutrition, The Children's Hospital and Institute of Child Health
Design	Observation of MPS feature
Reference population size	Not reported
Number of patients with MPS IVA	6
Prevalence (number per 100,000 live births)	Not reported
Comments	Urine samples for GAGs levels and dried blood samples for enzyme analysis was collected and immediately couriered to a reference metabolic laboratory in neighboring country, as the facility is not available in Pakistan

Reference	Moammar et al. 2010
Type of Source	Literature (peer-reviewed journal)
Region	*Saudi Arabia*
Collection Year(s)	1983-2008
Case Definition	MPS IVA
Data collection method	Review of inborn errors of metabolism
Design	Review
Reference population size	165,530 life births; only Saudi Aramco Medical Services Organization employees and their dependents.
Number of patients with MPS IVA	6
Prevalence (number per 100,000 live births)	3.62
Comments	Diagnosis was made by enzyme activity in cultured skin fibroblasts, liver biopsy, or leukocytes.

Reference	Lee et al. 2012 Cho et al. 2014
Type of Source	Literature (peer-reviewed journal)
Region	*South Korea*
Collection Year(s)	1994-2013 2004-2010
Case Definition	MPS IVA
Data collection method	Enzyme assay and molecular analysis.
Design	Samsung Medical Center. Patients' charts were retrospectively reviewed for clinical features and previous medical history.
Reference population size	Not reported
Number of patients with MPS IVA	MPS IVA 10 MPS IVA 3 (Cho et al. 2014)
Prevalence (number per 100,000 live births)	0.13
Comments	• Diagnosis was made by enzyme activity in leukocytes.

Reference	Lin et al. 2020 Chien et al. 2020 Lin et al. 2009
Type of Source	Literature (peer-reviewed journal)
Region	*Taiwan*
Collection Year(s)	1985-2019 2018-2019
Case Definition	MPS IVA
Data collection method	Clinical indication and newborn screening
Design	Screening
Reference population size	Not reported
Number of patients with MPS IVA	32 6 Chien et al. 2020
Prevalence (number per 100,000 live births)	0.33
Comments	• The diagnosis of the type of MPS was confirmed by a specific enzyme activity assay in serum, leukocytes and/or skin fibroblasts, • Two-dimensional electrophoresis of urinary GAGs, • Quantitative determination of DS, HS, KS, and CS using liquid chromatography-mass spectrometry (LC–MS/MS), and/or identification of the pathogenic mutations • newborn screening of MPS 4A and other LSDs were made possible by the 8-plex LSD screening assay; Chien et al. 2020.

Reference	Emre et al. 2002
Type of Source	Literature (peer-reviewed journal)
Region	*Turkey*
Collection Year(s)	Past 20 years
Case Definition	MPS IVA
Data collection method	118 patients suspected to have LSD from all over Turkey
Design	Survey
Reference population size	42 patients clinically diagnosed with MPS
Number of patients with MPS IVA	11
Birth Incidence (number of cases per 100,000 live births)	Not calculated
Comments	• Clinical diagnosis, blood and urine samples • Data not related to demographic data • High consanguinity rate in the population • MPS IVA % of MPS: 32.35

Reference	Al-Jasmi et al. 2012
Type of Source	Literature (peer-reviewed journal)
Region	*United Arab Emirates*
Collection Year(s)	1995-2010
Case Definition	MPS IVA
Data collection method	Review of LSD in Emiratis and non-Emiratis
Design	Review
Reference population size	Not reported
Number of patients with MPS IVA	2
Prevalence (number per 100,000 live births)	1.41
Comments	The diagnosis was made by clinical presentation and biochemical analysis

Reference	Applegarth et al. 2000
Type of Source	Literature (peer-reviewed journal)
Region	*Canada (British Columbia)*
Collection Year(s)	1972-1996
Case Definition	MPS IVA
Data collection method	Total number of cases diagnosed divided by total number of births
Design	Survey
Reference population size	Total births 1972-1996: 1,035,816 (British Columbia Vital Statistics Agency)
Number of patients with MPS IVA	5 (1 prenatal diagnosis)
Prevalence (number per 100,000 live births)	1.9
Comments	• Predominantly Caucasian population • Diagnoses confirmed by both laboratory and clinical investigations • Birth incidence for MPS: 1.9

Reference	Puckett et al 2021
Type of Source	Literature (peer-reviewed journal)
Region	*The United States*
Collection Year(s)	1995–2015
Case Definition	MPS IVA
Data collection method	The retrospective period used for the incidence study covered the period from 1995 to 2021. Prevalence was derived from the number of MPS patients alive.
Design	Survey
Reference population size	Total births 1995-2015: 80,118,336 (National MPS Society database records)
Number of patients with MPS IVA	87
Prevalence (number per 100,000 live births)	0.11
Comments	The number of MPS patients registered with the National MPS Society was used, no information of how they were diagnosed.

Reference	Josahkian et al 2020
Type of Source	Literature (peer-reviewed journal)
Region	*Brazil*
Collection Year(s)	1982-2019
Case Definition	MPS IVA
Data collection method	The birth prevalence was used to refer to the number of MPS cases diagnosed by the total number of live births in a specific period expressed as cases per 100,000 live births.
Design	Survey
Reference population size	Total births 1982-2019: 74,215,086 (The Brazilian Health System database)
Number of patients with MPS IVA	198
Prevalence (number per 100,000 live births)	0.15
Comments	MPS cases were diagnosed biochemically; the investigation frequently starts with a quantitative (colorimetric method with dimethylene blue) and qualitative (electrophoresis) analysis of urinary GAGs, followed by specific enzyme assays according to the first results and/or by identification of pathogenic variants by molecular genetic analysis.

Reference	Gomez et al 2012
Type of Source	Literature (peer-reviewed journal)
Region	*Colombia*
Collection Year(s)	1998-2007
Case Definition	MPS IVA
Data collection method	Patient records from different referral institutions for genetic diseases were reviewed, as well as live birth records between 1998 and 2007
Design	Survey
Reference population size	Total births 1998-2007: 1,767,059
Number of patients with MPS IVA	12 (MPS IV, not specified as A or B)
Prevalence (number per 100,000 live births)	0.68
Comments	In all clinical records, the assessment by a doctor specialized in Clinical Genetics was corroborated. The diagnosis was confirmed by glycosaminoglycan electrophoresis in urine, an assay to determine enzyme levels in leukocytes performed in specialized laboratories, or by both techniques, widely used in clinical practice for the diagnosis of mucopolysaccharidosis.

Reference	Mendoza-Ruvalcaba et al 2020
Type of Source	Literature (peer-reviewed journal)
Region	*Mexico*
Collection Year(s)	2012-2017
Case Definition	MPS IVA
Data collection method	The retrospective period used for the incidence per live births study covered the period from 2012 to 2017.
Design	Survey
Reference population size	Total births 2012-2015: 2,448,695
Number of patients with MPS IVA	95
Prevalence (number per 100,000 live births)	1.1
Comments	The enzyme activity measurement in leukocytes

Reference	Nasrallah et al 2023
Type of Source	Literature (peer-reviewed journal)
Region	*Tunisia*
Collection Year(s)	1999-2021
Case Definition	MPS IVA
Data collection method	Patients diagnosed with a MPS disorder in two referral laboratories in Tunisia between 1999 and 2021 were included.
Design	Survey
Reference population size	Total births 1999-2021: 3,822,983 (The National Bureau of Statistics of Tunisia)
Number of patients with MPS IVA	34
Prevalence (number per 100,000 live births)	0.86
Comments	Diagnosis was based on clinical and radiological features and analysis of urinary glycosaminoglycans, and enzyme assay in some of the patients