

# Macrophage Involvement in Mitral Valve Pathology in Mucopolysaccharidosis Type VI (Maroteaux–Lamy Syndrome)

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Maroteaux–Lamy syndrome (mucopolysaccharidosis type VI) is a rare lysosomal storage disorder in which the pathologic storage of glycosaminoglycans in various tissues can lead to severe symptoms, including cardiomyopathy. We report on a child with Maroteaux–Lamy syndrome whose cardiac condition deteriorated and eventually led to cardiac failure at the age of 7 years due to severe mitral regurgitation. She received a mitral valve replacement and tricuspid repair with successful outcome. Histologic examination of the mitral valve showed abundant “clear” cells in both the leaflets and chordae tendineae. In Hurler disease (MPS I), similar cells have been identified as activated valvular interstitial cells (VICs, a myofibroblast like cell type). Here we report that the “clear” cells are CD68 positive, a frequently used marker of macrophage lineage. The “clear” cells remained unstained with the more specific macrophage marker CD14 while persistent staining of other cells demonstrated macrophage infiltration. From these observations, we infer that macrophages are involved in mitral valve pathology in MPS VI. © 2013 Wiley Periodicals, Inc.

**Key words:** mucopolysaccharidosis; lysosomal storage disorder; heart valve pathology; mitral valve replacement; CD68; CD14

## INTRODUCTION

The mucopolysaccharidoses (MPSs) are a group of lysosomal storage disorders caused by the deficiency of a specific enzyme required for the stepwise degradation of glycosaminoglycans (GAGs), also known as mucopolysaccharides [Neufeld and Muenzer, 2001]. In mucopolysaccharidosis type VI (MPS VI), Maroteaux–Lamy syndrome (OMIM 253200), the lysosomal enzyme *N*-acetyl galactosamine 4-sulfatase (arylsulfatase B) is deficient and leads to accumulation of dermatan sulfate. The clinical hallmarks of MPS VI include profound skeletal deformities, short stature, hearing loss, corneal clouding, restrictive pulmonary

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function, and cardiac anomalies. The severity of the disorder varies considerably between patients [Valayannopoulos et al., 2010]. Enzyme-replacement therapy is available for MPS VI with positive results on pulmonary function, endurance, urinary GAG excretion, and cardiac dimensions [Harmatz et al., 2006; Braunlin et al., 2013].

We recently showed that severe cardiac features such as mitral valve regurgitation and dilated cardiomyopathy can be the key presenting symptom in children under 5 years old [Brands et al., 2013a]. We report on a severely affected MPS VI child who received a mitral valve replacement. The tissue specimens that were collected during the procedure enabled us to further elaborate on the pathophysiology of valve dysfunction in MPS VI.

Conflict of interest: none.

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## CLINICAL REPORT

A 3-year-old girl was referred to our hospital with severe mitral regurgitation, a dysmorphic mitral valve, moderate tricuspid regurgitation, and mild aortic regurgitation. Besides her cardiac problems, she had profound skeletal deformities with small stature, corneal clouding, and hepatosplenomegaly. On the basis of the cardiologic abnormalities and her coarse facial features, she was suspected to have Maroteaux–Lamy syndrome. She was born to consanguineous Pakistani parents. Both of the parents were heterozygous for a novel pathogenic splice site mutation c.1142 + 2 T>C (exon 5) in the *ARSB* gene. The patient was found to be homozygous for this mutation concordant with a deficiency of arylsulfate B activity in cultured skin fibroblasts (84.8 nmol/h mg in the patient vs. 379–980 nmol/h mg in healthy controls).

Over the next 3 years, the patient's mitral regurgitation and congestive heart disease worsened. Furosemide, spironolactone, captopril, and carvedilol were used as heart-failure medications. At the age of 6 years she became the first patient in the Netherlands to receive weekly enzyme replacement therapy (ERT) for MPS VI. After a few months her fatigue improved and she was more flexible in her joints. Even though she missed only two infusions in this period, her cardiac parameters did not improve: the dilatation of the left side of the heart and the valvular regurgitation progressed. The mitral valve leaflets showed no co-optation and had thickened further. The anterior mitral valve leaflet showed moderate prolapse. Figure 1 shows the severely enlarged left atrium and left ventricle, and severe mitral regurgitation on cardiac MRI.

At the age of 7 years she presented with life-threatening progressive heart failure, probably triggered by an upper airway infection. She became oxygen dependent and could not be stabilized with

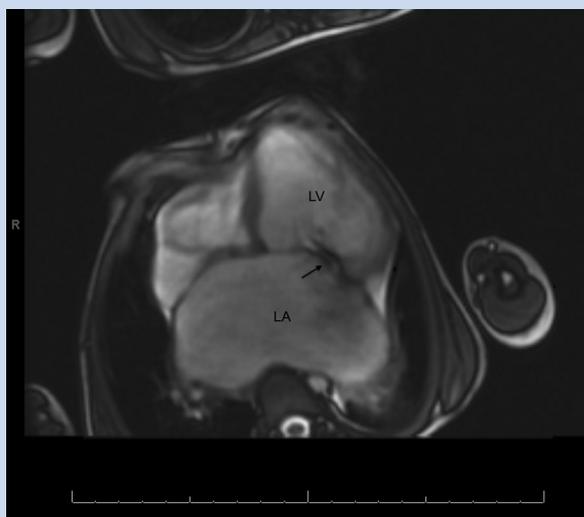
additional cardiac medication. As there were no alternatives, it was decided to intervene surgically. The mitral valve was replaced by a 25 mm St. Jude mechanical prosthesis, and tricuspid valve repair was performed. After surgery, she recovered well: the left ventricular end-diastolic diameter (LVEDD) decreased from 11.2 to 3.4 (Z-score), the left ventricular end-systolic diameter (LVESD) decreased from 12 to 3.7 (Z-score; as measured by M-mode echocardiography), and the right ventricular pressure normalized (see video in Supporting Information online).

The surgically removed valve displayed prominent abnormalities. Macroscopically, the leaflets were thickened and the architecture distorted. Microscopically, there were numerous vacuolated fibroblasts, also called “clear” cells or “Hurler” cells. They were mainly present in the rather loose connective tissue of the leaflets and in the dense connective tissue of the chordae tendineae, and less so in the adjacent cardiac muscle tissue of the chordae insertion sites (Fig. 2). The CD68 marker of macrophage lineage showed strong staining of macrophages that were spread throughout the tissue as well as diffuse staining of the “clear” cells (Fig. 2, panel E). CD68 is a member of the lysosomal-associated membrane glycoprotein family (LAMP), thus stains the GAG-loaded lysosomes of the “clear” cells. To distinguish the macrophages from the “clear” cells we subsequently applied the more specific macrophage marker CD14, whereby the “clear” cells were negative while the macrophages were positive (Fig. 2, panel F).

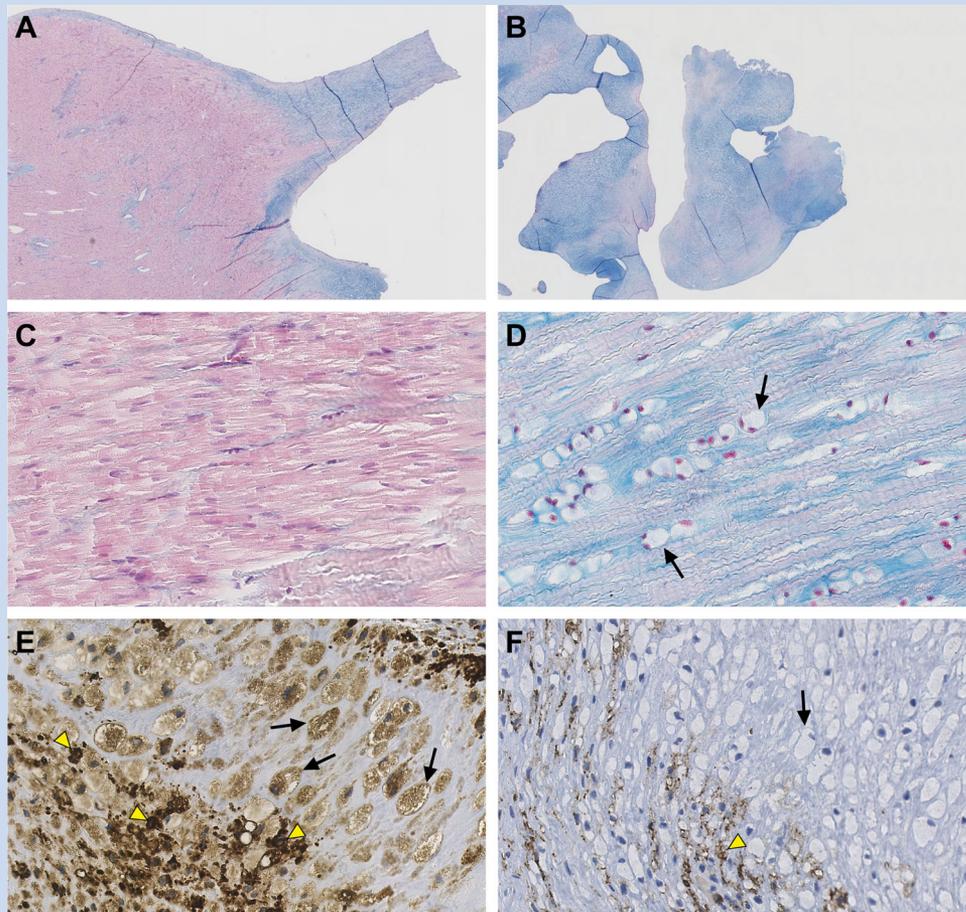
## DISCUSSION

Heart valve regurgitation is common in MPS VI, and the mitral valve seems to be most susceptible [Wippermann et al., 1995; Golda et al., 2012]. In healthy subjects, dermatan sulfate is the dominant GAG in heart valve connective tissue matrix [Grande-Allen et al., 2004]. It has therefore been speculated that in MPS types wherein dermatan sulfate accumulates (MPS I, II, VI and VII), the cardiac abnormalities are more severe than in other types of MPS [Dangel, 1998; Leal et al., 2010]. The cardiac problems of this reported patient manifested at a very young age and became life-threatening. The early onset and severity of symptoms are likely explained by the severity of the novel splice site mutation c.1142 + 2T>C [Mount, 1982; Brands et al., 2013b].

Histologic examination of the heart valve provided insight in the pathophysiology of MPS VI cardiac valvular disease. The “clear” cells that were abundantly present in the leaflets and the chordae tendineae were first described by Renteria et al. [1976] in a case of Hurler syndrome. Recently, they were demonstrated to be activated VICs (myofibroblasts) expressing vimentin and smooth muscle actin [Braunlin et al., 2011]. Using the macrophage lineage marker CD68 to visualize infiltration of pathologic tissue specimens, we unexpectedly observed staining of almost all cells in the valve leaflet (Fig. 2E). This can be explained by the CD68 protein being a member of the lysosomal-associated membrane protein family (LAMP) that would be expected to be abundant in “clear” cells that are packed with GAG-loaded lysosomes [Kostich et al., 2000; Grande-Allen et al., 2004]. Using CD14 (directed against a cell surface protein of macrophage lineage) as alternative and more specific marker of macrophage lineage [Amador-Ortiz et al., 2011] we were able to distinguish macrophage infiltration from GAG



**FIG. 1.** Magnetic resonance image [4-chamber view; obtained with steady-state free-precession MR sequence]. Note thickened mitral valve leaflets [arrow], massively enlarged left atrium (LA) and enlarged left ventricle (LV) resulting from mitral regurgitation.



**FIG. 2.** Microscopic images of the surgically resected mitral valve. Panels A,B: Junction of the chordae tendineae and the contractile cardiac tissue (panel A), and pieces of the valve leaflets (panel B; overview: alcian blue staining, original magnification 12.5 $\times$ ). Panels C,D: Area with cardiomyocytes in panel C and the presence of “clear” cells in the chordae tendineae in panel D (alcian blue staining, original magnification 200 $\times$ ). Panel E: Staining of both the “clear” cells (arrows) as well the macrophages (arrow heads) in the valve leaflet with antibody marker CD68 (200 $\times$  magnification), while panel F (200 $\times$  magnification) shows staining of only the macrophages (arrow heads), but not the fibroblasts (arrows) in the same area with antibody marker CD14.

storage in “clear” cells (activated VICs). Our results are at variance with those of Braunlin et al. [2011] who did not obtain CD68 staining in atrioventricular valves of infants with MPS I despite very similar valve pathology characterized by abundant “clear cells” that they characterized as activated VICs.

Based on the combination of findings, it is plausible that glycosaminoglycans accumulate in the lysosomes of VICs that synthesize and recycle GAGs, eventually depriving these cells of the capacity to maintain the valvular matrix. The ensuing tissue damage attracts macrophages that cause even more damage by lack of repair processes due to dysfunctional VICs. The presence of macrophages supports the theory that GAG storage induces inflammation through activation of the Toll-like receptor 4 signaling pathway [Simonaro et al., 2008, 2010]. Over time, the normal configuration of the heart valves is lost and valve regurgitation occurs.

In the case of this patient, treatment with ERT (recombinant human *N*-acetylgalactosamine-4-sulfatase, galsulfase) led to an

improvement of her clinical condition, which favored surgical treatment of the heart failure. Several studies have shown that ERT in MPS VI positively affects endurance, joint range of motion, and pulmonary function [Harmatz et al., 2008]. Though the disease process seems to halt, the cardiac valves are rather refractory to ERT even though the structures are surrounded by blood and freely exposed to galsulfase upon intravenous infusion [Braunlin et al., 2013], thus diffusion of galsulfase through the valvular tissue must be limited [Misfeld and Sievers, 2007]. The microscopic sections from this patient’s mitral valve confirm that significant amounts of GAGs were still present after 12 months of ERT using recommended dosing schedules per protocol.

Usually, cardiac surgeons refrain from valve replacement in severely affected children with MPS VI because of the poor prognosis. The successful outcome of mitral valve replacement and tricuspid repair in this case is an important experience that may guide others in the treatment of MPS VI and related MPS disorders.

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## REFERENCES

- Amador-Ortiz C, Hurley MY, Ghahramani GK, Frisch S, Klco JM, Lind AC, Nguyen TT, Hassan A, Kreisel FH, Frater JL. 2011. Use of classic and novel immunohistochemical markers in the diagnosis of cutaneous myeloid sarcoma. *J Cutan Pathol* 38:945–953.
- Brands MM, Frohn-Mulder IM, Hagemans ML, Hop WC, Oussoren E, Helbing WA, van der Ploeg AT. 2013a. Mucopolysaccharidosis: Cardiologic features and effects of enzyme-replacement therapy in 24 children with MPS I, II and VI. *J Inherit Metab Dis* 36:227–234.
- Brands MM, Hoogeveen-Westerveld M, Kroos MA, Nobel W, Ruijter GJ, Ozkan L, Plug I, Grinberg D, Vilageliu L, Halley DJ, Ploeg AT, Reuser AJ. 2013b. Mucopolysaccharidosis type VI phenotypes—genotypes and antibody response to galsulfase. *Orphanet J Rare Dis* 8:51–60.
- Braunlin E, Tolar J, Mackey-Bojack S, Masinde T, Krivit W, Schoen FJ. 2011. Clear cells in the atrioventricular valves of infants with severe human mucopolysaccharidosis (Hurler syndrome) are activated valvular interstitial cells. *Cardiovasc Pathol* 20:315–321.
- Braunlin E, Rosenfeld H, Kampmann C, Johnson J, Beck M, Giugliani R, Guffon N, Ketteridge D, Sá Miranda CM, Scarpa M, Schwartz IV, Leão Teles E, Wraith JE, Barrios P, Dias da Silva E, Kurio G, Richardson M, Gildengorin G, Hopwood JJ, Imperiale M, Schatz A, Decker C, Harmatz P, MPS VI Study Group. 2013. Enzyme replacement therapy for mucopolysaccharidosis VI: Long-term cardiac effects of galsulfase (Naglazyme ()) therapy. *J Inherit Metab Dis* 36:385–394.
- Dangel JH. 1998. Cardiovascular changes in children with mucopolysaccharide storage diseases and related disorders—Clinical and echocardiographic findings in 64 patients. *Eur J Pediatr* 157:534–538.
- Golda A, Jurecka A, Tyłki-Szymanska A. 2012. Cardiovascular manifestations of mucopolysaccharidosis type VI (Maroteaux–Lamy syndrome). *Int J Cardiol* 158:6–11.
- Grande-Allen KJ, Calabro A, Gupta V, Wight TN, Hascall VC, Vesely I. 2004. Glycosaminoglycans and proteoglycans in normal mitral valve leaflets and chordae: Association with regions of tensile and compressive loading. *Glycobiology* 14:621–633.
- Harmatz P, Giugliani R, Schwartz I, Guffon N, Teles EL, Miranda MC, Wraith JE, Beck M, Arash L, Scarpa M, Yu ZF, Wittes J, Berger KI, Newman MS, Lowe AM, Kakkis E, Swiedler SJ, MPS VI Phase 3 Study Group. 2006. Enzyme replacement therapy for mucopolysaccharidosis VI: A phase 3, randomized, double-blind, placebo-controlled, multinational study of recombinant human N-acetylgalactosamine 4-sulfatase (recombinant human arylsulfatase B or rhASB) and follow-on, open-label extension study. *J Pediatr* 148:533–539.
- Harmatz P, Giugliani R, Schwartz IV, Guffon N, Teles EL, Miranda MC, Wraith JE, Beck M, Arash L, Scarpa M, Ketteridge D, Hopwood JJ, Plecko B, Steiner R, Whitley CB, Kaplan P, Yu ZF, Swiedler SJ, Decker C, MPS VI Study Group. 2008. Long-term follow-up of endurance and safety outcomes during enzyme replacement therapy for mucopolysaccharidosis VI: Final results of three clinical studies of recombinant human N-acetylgalactosamine 4-sulfatase. *Mol Genet Metab* 94:469–475.
- Kostich M, Fire A, Fambrough DM. 2000. Identification and molecular-genetic characterization of a LAMP/CD68-like protein from *Caenorhabditis elegans*. *J Cell Sci* 113:2595–2606.
- Leal GN, de Paula AC, Leone C, Kim CA. 2010. Echocardiographic study of paediatric patients with mucopolysaccharidosis. *Cardiol Young* 20:254–261.
- Misfeld M, Sievers HH. 2007. Heart valve macro- and microstructure. *Philos Trans R Soc Lond B Biol Sci* 362:1421–1436.
- Mount SM. 1982. A catalogue of splice junction sequences. *Nucleic Acids Res* 10:459–472.
- Neufeld EF, Muenzer J. 2001. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Valle D, Sly W, Childs B, Kinzler KW, Vogelstein B, editors. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill Professional. pp 3421–3452.
- Renteria VG, Ferrans VJ, Roberts WC. 1976. The heart in the hurler syndrome: Gross, histologic and ultrastructural observations in five necropsy cases. *Am J Cardiol* 38:487–501.
- Simonaro CM, D’Angelo M, He X, Eliyahu E, Shtraizent N, Haskins ME, Schuchman EH. 2008. Mechanism of glycosaminoglycan-mediated bone and joint disease: Implications for the mucopolysaccharidoses and other connective tissue diseases. *Am J Pathol* 172:112–122.
- Simonaro CM, Ge Y, Eliyahu E, He X, Jepsen KJ, Schuchman EH. 2010. Involvement of the Toll-like receptor 4 pathway and use of TNF-alpha antagonists for treatment of the mucopolysaccharidoses. *Proc Natl Acad Sci USA* 107:222–227.
- Valayannopoulos V, Nicely H, Harmatz P, Turbeville S. 2010. Mucopolysaccharidosis VI. *Orphanet J Rare Dis* 5:5–24.
- Wippermann CF, Beck M, Schranz D, Huth R, Michel-Behnke I, Jungst BK. 1995. Mitral and aortic regurgitation in 84 patients with mucopolysaccharidoses. *Eur J Pediatr* 154:98–101.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s web-site.

**Movie S1.** M-mode echocardiogram. **Left panel:** echocardiogram prior to surgery with a severely dilated left atrium and dysmorphic mitral valve. **Right panel:** recent echocardiogram with a moderately dilated left atrium. Exact evaluation of the left atrial size is hampered by shadows of the mitral graft. Note the remarkable difference between left atrial size before and after mitral valve replacement.