

Gene Therapy Ameliorates Cardiovascular Disease in Dogs With Mucopolysaccharidosis VII

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Background—Mucopolysaccharidosis VII (MPS VII) is a lysosomal storage disease caused by deficient β -glucuronidase (GUSB) activity resulting in defective catabolism of glycosaminoglycans (GAGs). Cardiac disease is a major cause of death in MPS VII because of accumulation of GAGs in cardiovascular cells. Manifestations include cardiomyopathy, mitral and aortic valve thickening, and aortic root dilation and may cause death in the early months of life or may be compatible with a fairly normal lifespan. We previously reported that neonatal administration of a retroviral vector (RV) resulted in transduction of hepatocytes, which secreted GUSB into the blood and could be taken up by cells throughout the body. The goal of this study was to evaluate the effect on cardiac disease.

Methods and Results—Six MPS VII dogs were treated intravenously with an RV-expressing canine GUSB. Echocardiographic parameters, cardiovascular lesions, and biochemical parameters of these dogs were compared with those of normal and untreated MPS VII dogs.

Conclusions—RV-treated dogs were markedly improved compared with untreated MPS VII dogs. Most RV-treated MPS VII dogs had mild or moderate mitral regurgitation at 4 to 5 months after birth, which improved or disappeared when evaluated at 9 to 11 and at 24 months. Similarly, mitral valve thickening present early in some animals disappeared over time, whereas aortic dilation and aortic valve thickening were absent at all times. Both myocardium and aorta had significant levels of GUSB and reduction in GAGs. (*Circulation*. 2004;110:815-820.)

Key Words: cardiovascular diseases ■ gene therapy ■ lysosomes ■ mucopolysaccharidosis

The mucopolysaccharidoses (MPS) are a family of lysosomal storage diseases resulting from defective catabolism of glycosaminoglycans (GAGs) by 1 of 11 enzymes.^{1,2} In humans, the most common cardiovascular lesion, regardless of MPS type, is thickening of the mitral valve with regurgitation or stenosis.³ Aortic valve thickening and hypertrophic cardiomyopathy are the next most common lesions, with endocardial thickening and dilated cardiomyopathy also recognized.³ GAGs accumulate in the valve leaflets, with secondary fibrosis and nodular deformation. Primary myocardial involvement and infiltration of the coronary arteries with GAGs can also occur.⁴ Cardiac involvement is present in most patients with MPS, and the lesions are progressive, with risk of death as a result of congestive heart failure.³

A colony of MPS VII (β -glucuronidase [GUSB]-deficient) dogs⁵ have a mutation resulting in a single amino acid substitution, and cardiac abnormalities in affected dogs have been reported previously.^{6,7}

Enzyme replacement therapy, the intravenous injection of normal enzyme,⁸ has been successful in MPS VI mice, MPS I

dogs⁹ and cats,¹⁰ and human MPS I patients¹¹; however, cardiac function was not specifically evaluated.¹² Bone marrow transplantation (BMT) has been effective in MPS VII dogs⁶ and also appeared to be beneficial in some MPS I, IV, and VI children.^{13,14} Cardiac lysosomal storage was reduced in the MPS VII mouse after BMT, and GUSB activity was 53% of normal.^{15,16} A gene transfer experiment in the MPS VII mouse revealed that by 16 weeks of age, there was histochemical evidence of GUSB throughout the myocardium and a significant reduction in histological vacuolation in cardiac valves.¹⁷

The present report describes the detailed, long-term follow-up and histopathology of the cardiovascular features of neonatal dogs with MPS VII treated with intravenous retroviral vector (RV) gene therapy.⁷ We previously reported improved echocardiographic findings at 9 months after birth.

Methods

Animals

Dogs were raised under National Institutes of Health and US Department of Agriculture guidelines for the care and use of animals

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in research. Six MPS VII dogs (M1287, M1312, M1328, M1332, M1337, and M1339) had 20 mL of an RV containing the normal canine GUSB cDNA injected intravenously at 2 to 3 days of age as described previously.⁷ One dog (M1287) was pretreated with human hepatocyte growth factor (HGF) before injection of RV.⁷

Echocardiography

MPS VII-treated dogs were evaluated by physical examination and echocardiography with a Hewlett Packard 5500 Sonos ultrasound machine. All dogs were restrained in right and left lateral recumbency, with imaging performed from below by use of a cut-out table. The same, blinded sonographer (board-certified veterinary cardiologist) performed and scored all echocardiograms. 2D and M-mode echocardiography was performed with a 7.5- or 5-MHz probe. All valves were interrogated with color flow Doppler and pulse-wave Doppler using a 3.5-mHz transducer. A subjective score was assigned for mitral and aortic valve thickening, aortic root diameter (taken from the short-axis view at the level of the sinuses of Valsalva and at the level of the sinotubular junction from a long-axis plane), and quantity of valvular regurgitation. Results were evaluated by paired *t* test analysis.

Pathology

Euthanasia of dog M1312 was at 6 months of age with 80 mg/kg of sodium pentobarbital (Veterinary Laboratories, Inc). Dog M1339 died at 7 months of age while under propofol anesthesia. He appeared to be in good health before the procedure. The dogs were perfused with 2 L of cold saline before the collection of tissues, which were frozen immediately on dry ice or placed in buffered 10% formalin. Sections of paraffin-embedded aorta and myocardium 6 μ m thick were stained with hematoxylin and eosin. Histochemical staining with naphthol-AS-BI-beta-D-glucuronide for GUSB activity was performed on frozen sections as previously described.⁷

β -Glucuronidase and Total Hexosaminidase Assays

GUSB and total hexosaminidase A activities were measured by use of the respective fluorogenic substrates as described previously.^{6,18,19} Activity was expressed as nanomoles of 4-methylumbelliferone released per hour per milligram protein.

GAG Assays

Total sulfated GAGs were assayed by measurement of Alcian blue on the basis of the principles of Bjornsson²⁰ as described previously.⁹ Samples were normalized to protein concentrations (Biorad)²¹ and reported as micrograms total sulfated GAGs per milligram protein.

Results

We previously reported transduction of hepatocytes from 6 MPS VII dogs with an RV containing a normal copy of the canine GUSB cDNA after intravenous injection into newborns. The dogs had high levels of serum GUSB for 14 months after transfer,⁷ which have remained stable for more than 3 years (data not shown). No adverse clinical effects were evident from the time of the last report to the present.

Physical Examination and Echocardiography

Complete physical examinations and echocardiography were performed in untreated MPS VII, RV-treated MPS VII, and normal dogs. At 1 year of age, 3 of 4 untreated MPS VII dogs had murmurs of mitral valve regurgitation (MR), and all 4 dogs had echocardiographic evidence of MR, mitral valve thickening, and aortic root dilation. These results are consistent with our previous evaluation of 13 untreated MPS VII dogs 0.3 to 1.3 years old⁶ of which 9 of 13 had murmurs and all had mitral valve thickening, MR, aortic root dilation, and aortic valve thickening. The oldest untreated MPS VII dog

evaluated was only 1.3 years old, because MPS VII dogs undergo euthanasia because of the progression of skeletal deformity and an inability to stand or walk beyond 6 months of age. None of the untreated MPS VII dogs had clinical signs consistent with congestive heart failure.

Six RV-treated dogs were evaluated at 4 to 5 months after birth. Three of these dogs were also evaluated by echocardiography at 8 to 9 months and 24 months, and 2 were euthanized at 6 to 7 months. Mitral valves were mildly thickened in 4 (M1312, M1328, M1332, and M1339) of the 6 treated puppies at the time of initial evaluation (4 to 5 months of age). Mitral valve thickening was not detectable at the later times of evaluation (Figure 1). Insignificant MR was present by echocardiography in 2 (M1328 and M1332) of the 4 treated dogs evaluated at 9 to 11 months of age; in all 4, valve thickness was normal, and none of the dogs had murmurs. At 2 years of age, murmurs were still absent, and echocardiography showed normal mitral valve thickness and either minimal (in 1 dog, M1328) or no MR. The mild MR present in M1328 was consistent with the trivial valvular leaks that can often be found in normal dogs with close scrutiny of the valves by use of color flow Doppler. A similar amount of MR was detected in 3 of the 7 control dogs. The Data Supplement Movies demonstrate color flow Doppler echocardiograms at the mitral valve in an MPS VII dog, an RV-treated MPS VII dog, an HGF/RV-treated MPS VII dog, and an unaffected dog. The color flow Doppler jet of MR is present only in the untreated affected individual.

All RV-treated dogs evaluated had normal thickness of the tricuspid, aortic, and pulmonary valves and no aortic valve insufficiency, and the aortic diameter was subjectively within normal limits at all evaluations (Figure 2). Of 7 control dogs without MPS VII (6 to 38 months old), none had auscultatable murmurs on physical examination. However, 3 of the 7 had mild MR with no other abnormalities detected on echocardiography. All valves were subjectively of normal thickness.

β -Glucuronidase and Hexosaminidase Activity

Aortic root and left ventricular free wall GUSB activity was quantified. In 4 untreated MPS VII dogs, the GUSB in the aorta and myocardium was 0 ± 0 U/mg (0% of normal) and 0.50 ± 0.6 U/mg (0.5% of normal), respectively, as shown in Figure 3. In the 2 RV-treated MPS VII dogs, the mean GUSB activity in the aorta and myocardium was 16 U/mg (17.5% of normal) and 6 U/mg (19.3% of normal), respectively.

In many lysosomal storage diseases, levels of other lysosomal enzymes are elevated, and the activity falls to normal levels when lysosomal storage is reduced. The hexosaminidase A activity in untreated MPS VII dogs (Figure 3) was 6174 ± 2686 U/mg (1342% of normal) and 1556 ± 179 U/mg (416% of normal) for the aorta and myocardium, respectively. In the 2 RV-treated dogs, the hexosaminidase A activity was reduced to 1500 U/mg (326% of normal) and 681 U/mg (182% of normal) in the aorta and myocardium, respectively.

GAG Content

Untreated MPS VII dogs had 119 ± 34 μ g GAG/mg protein (365% of normal) and 2.5 ± 0.6 μ g GAG/mg protein (208%

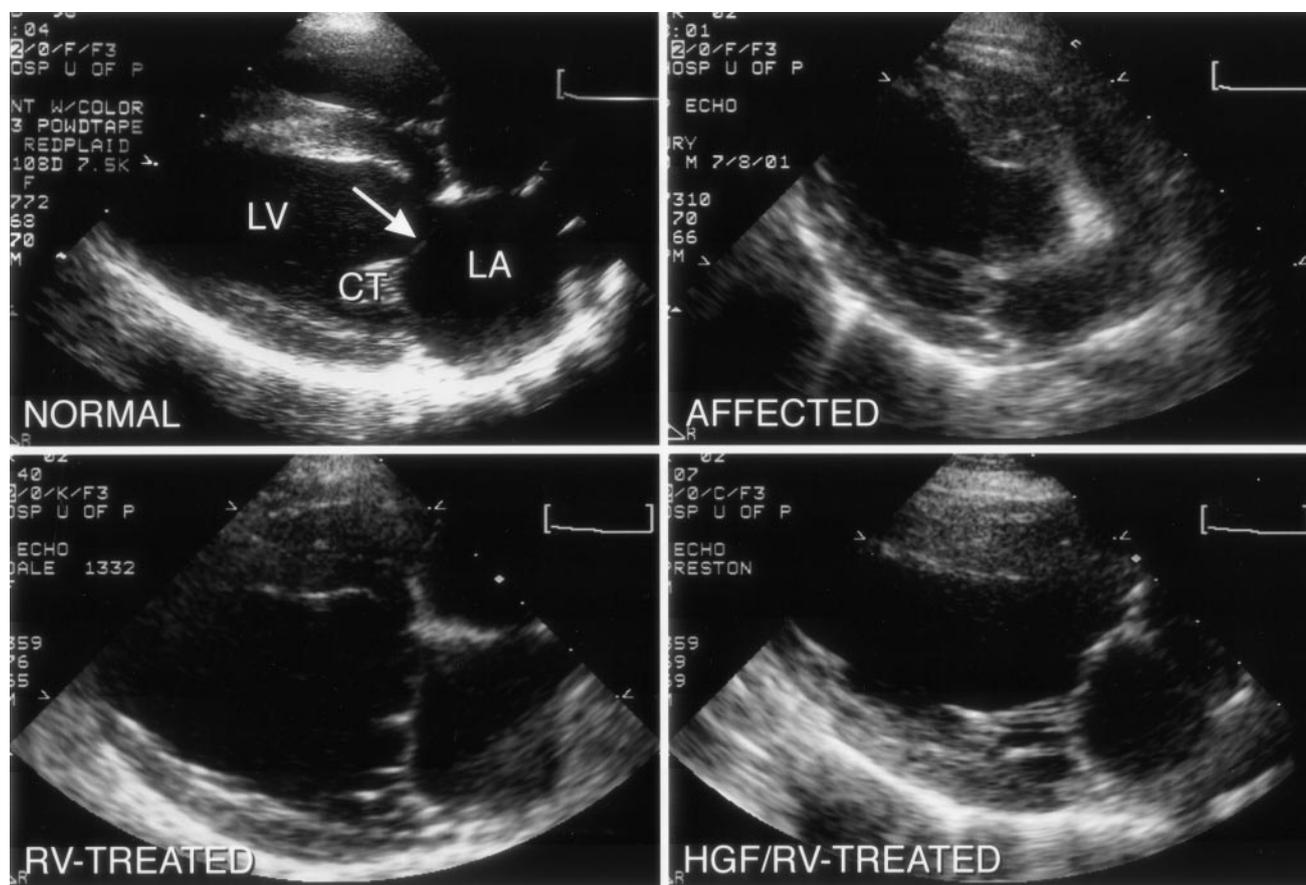


Figure 1. Canine echocardiograms. Long-axis right parasternal echocardiograms showing left atrium (LA), left ventricle (LV), mitral valve apparatus, and chordae tendineae (CT). A, Normal 4-month-old dog. Note thin valve leaflets and CT. B, Untreated 9-month-old MPS VII dog (M1390). Note thickened mitral valve leaflets and CT. C, Neonatal RV-treated dog (M1332) at 14 months. Valve thickness is normal. However, flattening of mitral valve annulus vs normal and HGF/RV-treated dogs is consistent with mild mitral valve prolapse. D, HGF/RV-treated dog (M1287) at 17 months. The valve appears anatomically normal.

of normal) in the aorta and myocardium, respectively (Figure 2). The average GAG levels in the RV-treated MPS VII dogs were reduced to $67.2 \mu\text{g}/\text{mg}$ protein (178% of normal) and $1.6 \mu\text{g}/\text{mg}$ protein (178% of normal) for aorta and myocardium, respectively.

Pathology

RV-treated MPS VII and control dogs were necropsied at 6 to 7 months of age. In the 2 untreated MPS VII littermates, the mitral valve had nodular thickening 3 to 4 mm in diameter, and the chordae tendineae were thickened (Figure 4B). In contrast, thickening of the mitral valves and chordae tendineae was mild in the RV-treated MPS VII dogs (Figure 4, C and D). Histologically, myocardial lesions in the untreated and RV-treated MPS VII dogs were minimal, with only a few rounded fibroblasts in the perivascular connective tissue with minimal to moderate hypertrophy and moderately vacuolated cytoplasm (data not shown). Histologically, the mitral valve nodules from the MPS VII-affected dogs consisted of loose connective tissue, with most fibroblasts severely hypertrophied with moderate to many cytoplasmic inclusions containing amorphous material, representing lysosomal storage. In 1 dog, subendocardial cells were severely hypertrophic, with highly vacuolated cytoplasm. Histologically, in the RV-

treated dogs, mild rounding of perivascular fibroblasts was present with minimal to moderate hypertrophy and cytoplasm with comparatively few large, pale pink vacuoles containing amorphous material.

Grossly, the aortic wall thickness, particularly the media, was increased in the untreated MPS VII dogs. In the RV-treated dogs, the aortic wall thickness was also increased, and in contrast to normal, the lumen was irregular. Histology of the untreated MPS VII dog aortas revealed that the normally fusiform smooth muscle cells of the media were rounded and severely hypertrophic, with highly vacuolated cytoplasm containing amorphous material (lysosomes distended with GAGs) (Figure 5, C and D). Histology of the aorta from the RV-treated dogs revealed that most smooth muscle cells in the media were fusiform and appeared normal, with few cells appearing rounded, with minimal to moderate hypertrophy and vacuolated cytoplasm (Figure 5, E through H).

In the RV-treated dogs, histochemical staining for GUSB activity showed enzyme activity in some cells throughout the entire thickness of the aortic wall, with most staining present in smooth muscle cells of the media (Figure 6C). GUSB activity was apparent in occasional cells in the myocardium (data not shown).

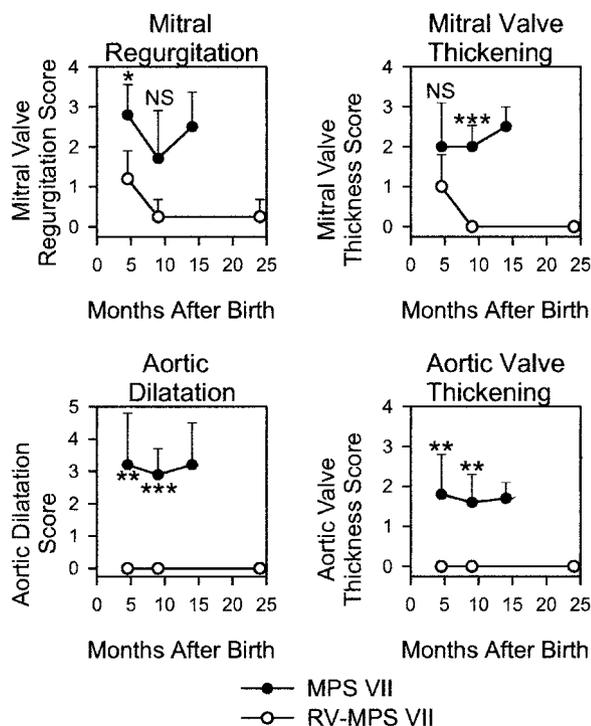


Figure 2. Evaluation of heart valves from untreated and RV-treated MPS VII dogs. Echocardiography was performed on untreated MPS VII (MPS VII) or RV-treated MPS VII (RV-MPS VII) dogs, and degree of MR, mitral valve thickening, aortic dilatation, and aortic valve thickening was determined by use of a scale in which 0 indicates normal and 1 to 4 indicate an abnormal examination of increasing severity. For untreated MPS VII dogs, evaluation involved 5 animals at 4 to 5 months after birth, 7 animals at 7 to 10 months, and 4 animals at 13 to 16 months. For RV-treated MPS VII dogs, evaluation involved 6 animals at 4 to 5 months after birth and 4 animals at 9 to 10 months and at 24 months. Fewer animals were evaluated at later times because of euthanasia of M1312 and M1339 at 6 months. Values for M1312 and M1339 at 4 to 5 months were similar to those of other RV-treated dogs at that time. Average values \pm SD are shown. Statistical comparisons were made between untreated MPS VII and RV-treated MPS VII dogs at early (4 to 5 month) and intermediate (7 to 10 month) times using Student's *t* test. * $P=0.05$ to 0.005 , ** $P=0.005$ to 0.0005 , *** $P<0.0005$, and NS indicates no significant difference between values for 2 groups at that time. Statistical comparisons were not performed for last set of values, because times of evaluation differed.

Discussion

Various MPS syndromes result in cardiovascular abnormalities, which may include valvular thickening, regurgitation and/or stenosis, and aortic dilation. Affected individuals can be treated by providing normal enzyme in 1 of 3 ways: (1) enzyme replacement therapy, (2) BMT, or (3) gene therapy. Enzyme replacement therapy and BMT have both been successful in reducing the cardiac manifestations of MPS in animals and humans.^{6,22–25} However, enzyme replacement therapy is expensive and requires frequent, lifelong administration, and BMT has traditionally resulted in substantial risks for patients,^{26,27} although recent development of nonablative marrow transplant with high cell dosage has proved to be effective and safer.¹⁶ One child with MPS VII has been studied after BMT therapy at 12 years of age. Mitral and aortic valve regurgitation did not change in the 15 months

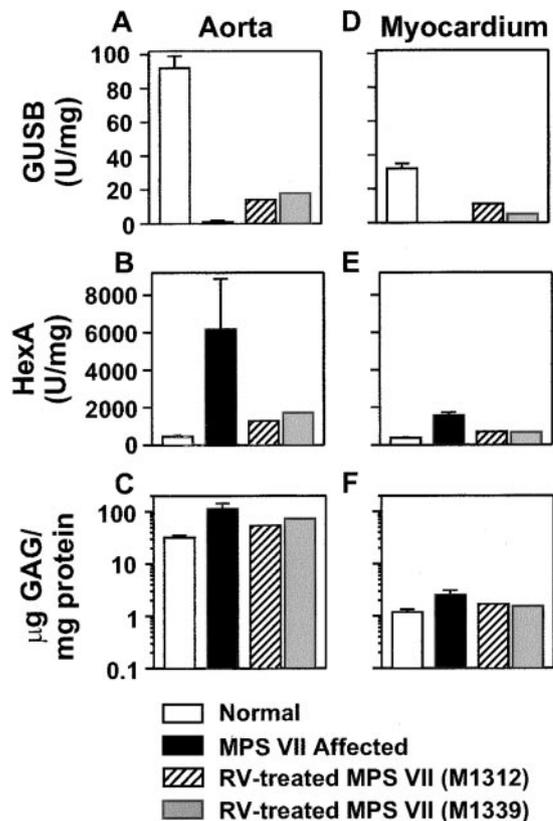


Figure 3. Analysis of GUSB, hexosaminidase A, and GAG levels in aorta and myocardium. All animals were euthanized at 6 to 7 months of age. Aorta and myocardium were collected from 4 normal dogs (Normal), 4 untreated MPS VII (MPS VII Affected), and 2 MPS VII dogs that were treated with RV shortly after birth (RV-treated MPS VII). Samples were homogenized, and GUSB activity, hexosaminidase A activity, and GAG levels were determined and normalized to amount of protein in sample and plotted as average \pm SD. Levels for aorta and myocardium are plotted on same scale. Hexosaminidase A is another lysosomal enzyme, levels of which are increased in untreated MPS VII dogs.

after therapy,²³ nor did valve abnormalities improve in a group of MPS I, IV, and VI children treated with BMT. The effect on the heart of gene therapy of hematopoietic cells in MPS VII mice^{19,28} was not reported.

The cardiovascular abnormalities seen by echocardiography in untreated MPS VII dogs in this and a previous study⁶ included thickening of the mitral valve with MR, large aortic dimensions in the short- and long-axis views, and thickening of the aortic valve. All of the 4 RV-treated dogs evaluated when older than 7 months of age had normal valve thickness, with valve anatomy that remained normal at 2 years of age, although 2 had mild MR when <1 year of age. The variation over time suggests that continued remodeling and improvement may occur during the 2 years after therapy.

Histologically, cardiovascular lesions were also dramatically improved in the heart valves and aorta of RV-treated dogs compared with untreated affected littermates.

The GUSB activity was increased in the myocardium and aorta of RV-treated MPS VII dogs, and the GAG content was reduced. The amount of normal enzyme activity needed to correct the metabolic defect and produce phenotypic correc-

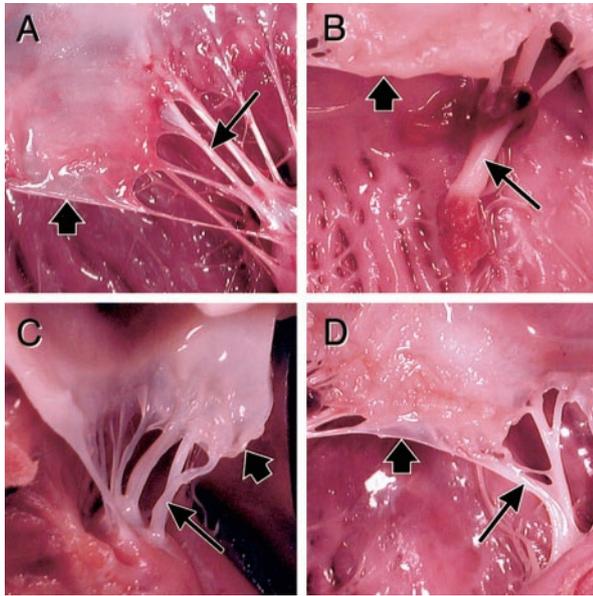


Figure 4. Gross evaluation of mitral heart valve. A, A 6-month-old normal dog; B, a 6-month-old untreated MPS VII dog; C and D, RV-treated MPS VII dogs at 6 and 7 months old (M1312 and M1339, respectively). Thick arrows show edge of septal mitral valve leaflet, and thin arrows show chordae tendineae. In untreated MPS VII dog, thickness of valve and chordae tendineae is increased vs normal (A), whereas lesions are less in treated dogs (C, D) vs untreated dog (B).

tion is only a few percent of normal.^{19,29} Although the 2 dogs were perfused with saline after death to remove blood containing GUSB, the procedure may have been imperfect, yielding higher tissue levels. Regardless, the amount of GUSB activity was sufficient to reduce the tissue GAG concentration, albeit not to normal levels. The lack of complete normalization of the cardiac GAG content is somewhat paradoxical, because BMT produced a reduction of GAG to normal levels in 3 BMT-treated dogs, although some residual signs of storage persisted on histopathological assessments.⁶

Optimal therapy aims to normalize cardiovascular structures or at least prevent the progressive anatomic deterioration that is often observed in the MPS syndromes. From the findings in this report, the cardiovascular lesions of MPS VII in dogs were ameliorated with neonatal intravenous RV gene therapy. Valve replacement has been performed successfully in humans with MPS IS,³⁰ MPS IIIB,³¹ and MPS IV³² when progressive deterioration necessitated therapy to prevent further progression of disease. Alternatively, medical management may control congestive heart failure. RV gene therapy appears to be superior to BMT, because a matched donor and pretransplant conditioning are not necessary. No adverse effects secondary to gene therapy have been detected thus far. However, because most children with lysosomal storage diseases are currently not diagnosed at birth, RV gene therapy will need to be evaluated in older MPS VII dogs to determine whether adequate transduction can be achieved and what minimal level of GUSB is necessary to control the cardiovascular disease.

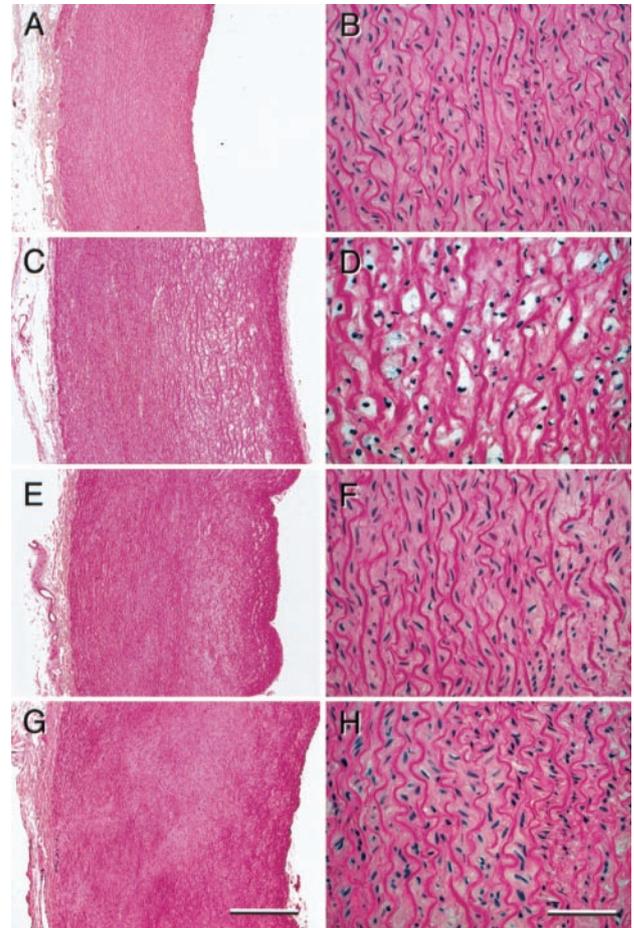


Figure 5. Pathology of aorta. Light micrographs of aortic wall of a 6-month-old normal dog (A, B), a 6-month-old untreated MPS VII dog (C, D), and RV-treated MPS VII dogs (E, F, and G, H; 6 and 7 months old, M1312 and M1339, respectively). Hematoxylin and eosin, magnification $\times 12.5$ (A, C, E, G; bar = $50 \mu\text{m}$) and $\times 62.5$ (B, D, F, H; bar = $10 \mu\text{m}$).

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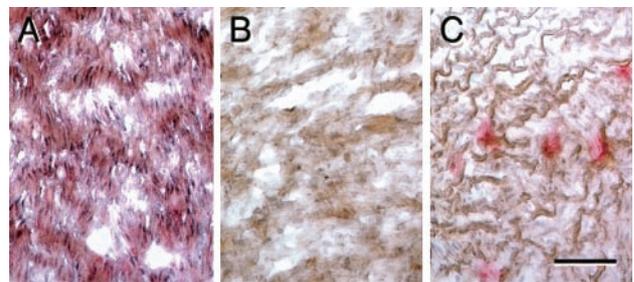


Figure 6. GUSB activity in aorta. Sections of aortas that were obtained at 6 to 7 months after birth were stained for GUSB activity, which appears red, counterstained with hematoxylin (blue for nuclei). Normal (A), untreated affected (B), and RV-treated (C; M1312) dogs. Bar = $50 \mu\text{m}$.

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