A Novel Model of Murine Mucopolysaccharidosis Type VII due to an Intracisternal A Particle Element Transposition into the β -Glucuronidase Gene: Clinical and Pathologic Findings

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ABSTRACT

We describe the clinical and pathologic findings in a murine model of mucopolysaccharidosis VII (Sly disease) that arose spontaneously in the C3H/HeOuJ mouse strain. Affected *gus*^{mps2J}/*gus*^{mps2J} mice are deficient in β -glucuronidase because of insertion of an intracisternal A particle element into intron 8 of the *gus* structural gene. This is the first model of a human lysosomal storage disease caused by an intracisternal A particle element insertion. Mice with the *gus*^{mps2J}/*gus*^{mps2J} genotype have <1% of normal β -glucuronidase activity and secondary elevations of other lysosomal enzymes. The phenotype includes shortened life-span, dysmorphic features, and skeletal dysplasia. Lysosomal storage of glycosaminoglycans is widespread and affects the brain, skeleton, eye, ear, heart valves, aorta, and the fixed tissue macrophage system. Thus the phenotypic and pathologic alterations in *gus*^{mps2J}/*gus*^{mps2J} mice are similar to those in patients with mucopolysaccharidosis VII. The finding of antibodies to β -glucuronidase in some older *gus*^{mps2J}/*gus*^{mps2J} mice suggests the mice produce sufficient enzyme to elicit an immune

response. The *gus*^{mps2J}/*gus*^{mps2J} model provides another well-defined genetic system for the study of the pathophysiology of mucopolysaccharidosis and for evaluation of experimental therapies for lysosomal storage diseases. The disease in *gus*^{mps2J}/*gus*^{mps2J} mice is less severe than that seen in the previously characterized B6.C-H2^{bm1}/ByBir-*gus*^{mps}/*gus*^{mps} mouse model. Furthermore, unlike *gus*^{mps}/*gus*^{mps} mice, *gus*^{mps2J}/*gus*^{mps2J} mice are fertile and breed to produce litters, all of which are mucopolysaccharidosis VII pups. This feature makes them extremely useful for testing intrauterine therapies. (*Pediatr Res* 49: 342–348, 2001)

Abbreviations:

MPS, mucopolysaccharidosis
LSD, lysosomal storage disease
GAG, glycosaminoglycan
GUSB, β -glucuronidase
IAP, intracisternal A particle

The MPSs are a group of LSDs characterized by a deficiency of a lysosomal acid hydrolase that degrades GAG. MPS patients with different enzyme deficiencies share clinical, pathologic, and biochemical features, including progressive lysosomal GAG accumulation and associated cellular and organ dysfunction. Premature death, mental retardation, coarse fa-

cies, and widespread skeletal and joint abnormalities, collectively referred to as dysostosis multiplex and hepatosplenomegaly, are seen in most patients with MPS (1). MPS VII (Sly disease) is characterized by GUSB (EC 3.2.1.31) deficiency and clinical and pathologic features similar to those seen in other types of MPS (1–3).

Effective therapies for patients with LSD are limited, and experiments on meaningful numbers of genetically identical mice with LSD can provide useful information on the effect of treatments for these disorders (4). Animal models of LSD also allow detailed study of the pathophysiology of these disorders. Several spontaneously occurring models of MPS have been described and used to test therapies (1, 4). The ideal model has clinical findings and pathology that resemble those of human LSD and is hardy enough to survive therapy.

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We identified earlier a small, dysmorphic C3H/HeOuJ male mouse homozygous for a spontaneous mutation, *gus*^{mps2J}, in the GUSB structural gene, which results in <1% normal GUSB activity (5). Affected offspring of the mouse have markedly reduced GUSB mRNA and secondary elevation of other lysosomal enzymes. The mutation is caused by a 5.4-kb IAP element insertion into intron 8 of the structural gene for GUSB on chromosome 5 (5). The IAP insertion correlates with a dramatic reduction in the amount of normal size *gus* mRNA and the presence of a truncated *gus* mRNA expressed at 5- to 7-fold less than normal (5). This is the first LSD model caused by insertion of an IAP into the structural gene for a lysosomal enzyme. Murine IAPs are defective retroviruses encoded by a family of endogenous proviral elements; their transposition into genes can alter transcription.

The *gus*^{mps2J}/*gus*^{mps2J} mice have a progressive clinical course and pathologic features, including shortened life span, skeletal dysostosis, and widespread lysosomal storage with marked lysosomal distention in the fixed tissue macrophage system and skeleton. These clinical and pathologic findings parallel those of humans with MPS. The phenotype of the *gus*^{mps2J}/*gus*^{mps2J} mouse is similar to, although less severe than, that seen in a previously described model of murine MPS VII (6, 7) with a 1-bp deletion in exon 10 of the *gus* gene (8).

METHODS

Clinical comparisons of C3H/HeOuJ-*gus*^{mps2J}/*gus*^{mps2J} affected mice and phenotypically normal *gus*^{mps2J}/+ mice maintained at The Jackson Laboratory were performed since 1992. All mice were handled with the highest standards of humane animal care. The Jackson Laboratory Animal Care and Use Committee approved the study we performed. This committee is the applicable institutional review board at The Jackson Laboratory. The Jackson Laboratory is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. The *gus*^{mps2J}/*gus*^{mps2J} mice were identified by measuring GUSB levels in tail tissue. Eighteen male and 26 female *gus*^{mps2J}/*gus*^{mps2J} mice born before 1995 and six male and 19 females born after 1994 were followed to determine survival. Survival was compared using the *t* test. Groups of phenotypically normal *gus*^{mps2J}/+ males (*n* = 22) and females (*n* = 7) and of *gus*^{mps2J}/*gus*^{mps2J} males (*n* = 14) and females (*n* = 14) were weighed at intervals from birth (0 d) to >1 mo of age.

Nineteen *gus*^{mps2J}/*gus*^{mps2J} and eight phenotypically normal mice from 1 to 19 mo old obtained before 1995 were killed by asphyxiation, and organs were sampled for histology. Tissue was fixed in 2% glutaraldehyde in 4% paraformaldehyde in PBS and postfixed in 1% osmium tetroxide. Samples of liver, spleen, kidney, skin, heart, aorta, bone and cartilage at the costochondral junction, and brain including hippocampus, parietal cortex and subcortical white matter, and cerebellum were prepared and studied by light or electron microscopy as previously described (7).

The skeletons, including dissected long bones and skulls, were radiographed using a Faxitron Radiographic Analyzer. (Hewlett Packard, McMinnville, Oregon, U.S.A.) Images of

forelimb bones of three normal and six *gus*^{mps2J}/*gus*^{mps2J} male adult mice were measured to evaluate long bone growth. The long bones from 3, 6, and 12 mo old mice were decalcified, routinely processed, and stained with hematoxylin and eosin and alcian blue-periodic acid-Schiff to identify tissue GAG histologically (7). The external and middle ears from the same mice were dissected, fixed in 4% neutral buffered formalin, decalcified, and sectioned as previously described (9).

Eighteen *gus*^{mps2J}/*gus*^{mps2J} mice >1 y (16 maintained by M.S.S., two maintained by E.H.B.) and eight *gus*^{mps2J}/*gus*^{mps2J} mice ≤1 y of age maintained by J.B. were evaluated using an ELISA technique as previously described (10) for antibodies to GUSB. Samples of intestine, brain, and liver of 16 of the mice >1 y of age were frozen, sectioned, and stained with a histochemical technique to demonstrate GUSB activity (10).

RESULTS

The *gus*^{mps2J}/*gus*^{mps2J} mice were dysmorphic with growth retardation, a blunted facies, and short limbs (Fig. 1). Dysmorphic features were readily apparent in weanling mice. Male *gus*^{mps2J}/*gus*^{mps2J} mice weights were similar to normal from 1 to 21 d of age. At weaning, the affected males tended to weigh less than normal, and as they aged, the difference in weight was

Fig. 1. A 1-y-old *gus*^{mps2J}/*gus*^{mps2J} mouse (right) is easily distinguished from its littermate (left) by its small size, short blunted nose, short limbs, and thick tail.

maintained (Fig. 2). Females weighed less than males, and the affected females, like the affected males, weighed less than normal females after weaning. Average survival of 44 *gus^{mps2J}/gus^{mps2J}* mice born between 1992 and 1994 was 431 d (Fig. 3). For 25 mice, all of the same *gus^{mps2J}/gus^{mps2J}* strain, but born between 1995 and 1997, average life-span was 187 d. Females were, on average, shorter lived than males.

Radiographic alterations in skeletons of *gus^{mps2J}/gus^{mps2J}* mice included wide oar-shaped ribs, a shortened wide pelvis, broad, short limbs, particularly proximal long bones, and a generalized increase in bone radiodensity (Fig. 4). In adult *gus^{mps2J}/gus^{mps2J}* mice, the humerus was 85% of normal bone length and the radius-ulna, 94% of normal.

There was histologic evidence of lysosomal storage in all tissues examined, although the amount of storage varied from cell to cell and among mice. The fixed tissue macrophage system was affected with cytoplasmic vacuolization in Kupffer cells (Fig. 5A), spleen, and bone marrow sinus-lining cells. Ultrastructurally, the vacuoles had a single membrane, and the stored material was fine fibrillogranular with associated large electron dense granules (Fig. 5B). When the fibrillogranular material was lost in processing, vacuoles appeared empty. Hepatocytes did not have lysosomal storage. Spleen lymphocytes were only occasionally affected with a small amount of vacuolization.

Glomerular visceral and parietal epithelial cells and renal tubular epithelial cells had lysosomal distention. Skin storage was primarily in the dermal fibroblasts and in s.c. fat cells. The heart valves contained vacuolated stromal cells; scattered perivascular cells distended by vacuoles were seen in the myocardium, but myocytes contained no storage. Aortic medial and endothelial cells were distended by storage.

The ears of the *gus^{mps2J}/gus^{mps2J}* mice were markedly altered, showing changes similar to those previously described in the *gus^{mps}/gus^{mps}* MPS VII model (Fig. 6) (9). Several *gus^{mps2J}/gus^{mps2J}* mice had otitis media with associated thickening of the connective tissue of the middle ear mucosa. The tympanic membrane was thickened and ossicles were altered with vacuolization of chondrocytes and osteocytes and expansion of the osseous lacunae. Bone in other sites was also

Fig. 2. The weight of MPS VII males (■) and females (◆) tended to be less than that of phenotypically normal male (●) and female (▲) mice near weaning and thereafter.

Fig. 3. Survival of the *gus^{mps2J}/gus^{mps2J}* mice is shorter than that of normal mice. Phenotypically normal mice live approximately 604 d. The *gus^{mps2J}/gus^{mps2J}* colony had a mean survival of 431 d for mice [▲ females, 428 d $\pm$ 130 d (mean $\pm$ SEM); ◆ males, 476 d $\pm$ 132 d] born before 1995. Although of the same strain, *gus^{mps2J}/gus^{mps2J}* mice born during and after 1995 had a shorter mean survival of only 187 d (● females, 150 d $\pm$ 58 d; ■ males, 262 d $\pm$ 122 d). The cause of this change in survival over time (females, $p < 0.01$; males, $p = 0.002$) is unknown and was not observed in another colony housed outside of The Jackson Laboratory.

Fig. 4. The skull of a 1-y-old *gus^{mps2J}/gus^{mps2J}* mouse (right) is shorter with thickened broad zygomatic arch and diffuse increase in radiodensity compared with an age-matched phenotypically normal mouse (left). The long bones are thickened, broad, and short in the *gus^{mps2J}/gus^{mps2J}* mouse (right) compared with the same bones in a normal mouse (left).

abnormal with marked lysosomal distention in osteoblasts, osteocytes, and chondrocytes (Fig. 7). The synovium of large stifle joints was proliferated with marked stromal cell vacuolization and GAG accumulation.

The corneal fibroblasts in *gus^{mps2J}/gus^{mps2J}* mice had a moderate amount of cytoplasmic vacuolization, but retinal pigment epithelium had no lysosomal distention. Phenotypically normal mice of the C3H/HeOuJ strain, on which the *gus^{mps2J}* mutation arose, have photoreceptor degeneration, a finding also seen in the *gus^{mps2J}/gus^{mps2J}* mice of this strain.

In the CNS, lysosomal distention was seen in leptomeningeal, parenchymal perivascular, and glial cells and neocortical (Fig. 8A) and hippocampal (Fig. 8B) cortex neurons. The amount of lysosomal storage in different neuronal groups

Fig. 5. *A*, liver Kupffer cells from a 6-mo-old *gus*^{mps2J}/*gus*^{mps2J} mouse have lysosomal distention, which is variable in amount but apparent in all mice examined. Hepatocytes have no lysosomal distention (toluidine blue, ×543). *B*, ultrastructurally, *gus*^{mps2J}/*gus*^{mps2J} mice have Kupffer cells in the liver with marked lysosomal distention by fine fibrillogranular storage material as well as electron dense granules (uranyl acetate-lead citrate, ×2450).

varied among age-matched *gus*^{mps2J}/*gus*^{mps2J} mice and did not always correlate with the animal's age except in the hippocampus.

Before age 6 mo, the amount of storage throughout the hippocampus was sparse, without a clear gradient. After 6 mo, relative sparing of the pyramidal neurons in CA₁ sector of the hippocampal formation was apparent, but storage elsewhere in the hippocampus increased with age and was maximal at 1 y of age.

The cerebellar cortex Purkinje cells contained abundant electron-dense flocculent storage material (Fig. 8, *C* and *D*) similar to that previously described in another MPS VII model (11). Storage was not progressive after 3 mo of age. Meganeurites were prominent in the affected mice, appearing at 3 mo of age; they were most numerous in the oldest mice examined (14 and 19 mo of age) and occurred in both the hippocampal cortex and neocortex.

Seven of the 18 *gus*^{mps2J}/*gus*^{mps2J} mice >1 y of age had antibodies to GUSB detected by ELISA. None of the younger *gus*^{mps2J}/*gus*^{mps2J} mice had demonstrable antibody. GUSB was

not quantitated in tissue, but there were no GUSB-containing cells identified in the 16 *gus*^{mps2J}/*gus*^{mps2J} mice examined histochemically.

DISCUSSION

Marked phenotypic heterogeneity has been noted in human MPS VII (1). This report illustrates heterogeneity in murine MPS VII as well. The *gus*^{mps2J}/*gus*^{mps2J} mice have a less severe disease than that of the previously described *gus*^{mps}/*gus*^{mps} mice (7). The *gus*^{mps2J}/*gus*^{mps2J} mice are able to bear live young and raise them to weaning, whereas *gus*^{mps}/*gus*^{mps} mice conceive with difficulty, rarely carry litters to term (12), and do not lactate. Whether the milder phenotype of *gus*^{mps2J}/*gus*^{mps2J} mice is associated with comparably milder functional deficits than those observed in *gus*^{mps}/*gus*^{mps} mice (13–15) has not yet been evaluated.

When first observed, the *gus*^{mps2J}/*gus*^{mps2J} mice had a much longer average life-span (431 d) than *gus*^{mps}/*gus*^{mps} mice (139–170 d). Since 1995, an unexplained decrease in survival to an average of 187 d was noted for *gus*^{mps2J}/*gus*^{mps2J} mice maintained at The Jackson Laboratory (Fig. 3). An environmental change (*e.g.* handling, nutrition, infection) may have contributed to the recent change in survival of the *gus*^{mps2J}/*gus*^{mps2J} mice observed at The Jackson Laboratory. This change in survival of mice maintained at The Jackson Laboratory correlated with a change from Wayne Grain to National Institutes of Health-31 diet, but further investigations are necessary to determine what environmental factor, if any, has a role in the survival of the *gus*^{mps2J}/*gus*^{mps2J} strain. *gus*^{mps2J}/*gus*^{mps2J} mice maintained by M.S.S. at Washington University on a diet similar in fat content to Wayne Grain have an average life-span of approximately 1 y (data not shown).

The ultrastructural character of lysosomal GAG accumulation is similar in both MPS VII models, although, overall, lysosomal distention is less prominent in the *gus*^{mps2J}/*gus*^{mps2J} mice. The brain in the *gus*^{mps2J}/*gus*^{mps2J} mice has striking meganeurite formation, seen only occasionally in *gus*^{mps}/*gus*^{mps} mice (11). Meganeurites are structural alterations of the neuronal axon hillock–initial axon segment and contain undegraded substrate. They are described in both human and animal LSD (11) and are thought to interfere with cell function by altering electrical properties of neurons (16).

The relative lack of lysosomal storage in neurons in CA₁ of the hippocampus is similar to that seen in the *gus*^{mps}/*gus*^{mps} mice, and in humans with MPS VII and IV (11). The variability in amount of storage in neuronal groups may reflect site-specific differences in GUSB dependency for GAG turnover. Unlike *gus*^{mps}/*gus*^{mps} MPS VII mice, *gus*^{mps2J}/*gus*^{mps2J} mice have no retinal pigment epithelium storage. This may be related to the photoreceptor loss characteristic of the C3H strain. Retinal pigment epithelium has a role in outer segment photoreceptor turnover, and without photoreceptors, GUSB deficiency may not result in GAG storage.

The phenotypic differences between the two inbred mouse strains, *gus*^{mps2J}/*gus*^{mps2J} and *gus*^{mps}/*gus*^{mps}, may be the result of genetic modifiers on the C3H background that modulate expression of GUSB deficiency in *gus*^{mps2J}/*gus*^{mps2J} mice.

Fig. 6. *A*, ear of a 6-mo-old gus^{mps2J}/gus^{mps2J} mouse has laminated keratin in the external auditory canal (EC), a thick tympanic membrane, and otitis in the middle ear cavity (ME). *B*, same area of the EC and ME from a normal littermate is histologically normal. *C*, malleus (M) and incus (I) of the middle ear of a 3-mo-old gus^{mps2J}/gus^{mps2J} mouse has bone dysplasia similar to that seen in the other bones, with distention of osteocytes and chondrocytes by enlarged lysosomes and expansion of the lacunae. The ossicles are adhered to the adjacent middle ear mucosa. *D*, same ossicles from a phenotypically normal littermate have no alteration in the osteocytes or chondrocytes (hematoxylin and eosin; *A*, *B*: $\times 32$; *C*, *D*: $\times 84$).

Fig. 7. The cortical bone from the rib of a 1-mo-old gus^{mps2J}/gus^{mps2J} mouse has osteocyte and osteoblast vacuolization. The sinus lining cells in the adjacent bone marrow have minimal lysosomal distention (toluidine blue, $\times 543$).

Wild-type C3H mice have only 10% to 38% of the GUSB activity seen in wild-type B6 mice (5). The reduced GUSB activity may allow other pathways of GAG metabolism to develop in the C3H/HeOuJ mice, thus partially compensating for the marked reduction in GUSB. On the other hand, residual

GUSB activity in the gus^{mps2J}/gus^{mps2J} mouse kidney is 0.5% the level found in the C3H/HeOuJ $^{+/+}$ mice compared with 0.005% of wild-type B6 levels in gus^{mps}/gus^{mps} mice. Possibly, the greater residual activity moderates consequences of the deficiency and contributes to the milder phenotype. Breeding the gus^{mps} mutation onto the C3H/HeOuJ background (17) and the gus^{mps2J} mutation onto the B6 background could help distinguish the contribution to enzyme deficiency and strain-specific modifying genes on the different phenotypes.

The appearance of GUSB antibody in some animals was an unexpected and very interesting finding. It raises the possibility that the gus^{mps2J} gene presumably silenced by the IAP element insertion might be activated over time in some cells and produce enzyme that is recognized as foreign by the mouse, naïve to this antigen at birth. Consistent with this hypothesis is the observation by Gwynn *et al.* (5) that one of five gus^{mps2J}/gus^{mps2J} mice tested had an inexplicably high level of GUSB activity limited to the brain. What impact GUSB activity or antibodies to GUSB may have on survival of gus^{mps2J}/gus^{mps2J} mice requires further study. In long-term therapeutic studies using the gus^{mps2J}/gus^{mps2J} mice, evaluation of GUSB enzyme and antibody levels will be important.

Therapies for LSD in humans have included bone marrow transplantation and enzyme replacement (18). Animal models

Fig. 8. The adult gus^{mps2J}/gus^{mps2J} mice have widespread but mild neuronal lysosomal storage, here in a 1-y-old mouse cortex (A) and hippocampus (B). Meganeurites are prominent, particularly in mice >3 mo old. C and D, Purkinje cell from a 1-y-old gus^{mps2J}/gus^{mps2J} mouse contains flocculent lysosomal storage material (A, B: toluidine blue $\times 543$; C, D: uranyl acetate-lead citrate, C: $\times 4,402$, D: $\times 12,250$).

have provided useful information on potential therapies for LSD (1, 4). The gus^{mps2J}/gus^{mps2J} mice offer several advantages in evaluating effects of various therapies. Because the gus^{mps2J}/gus^{mps2J} mice breed, larger numbers of MPS VII mice can be produced from matings of homozygous parents than of heterozygous matings. In addition, because all pups in gus^{mps2J}/gus^{mps2J} matings are MPS VII (rather than one in four in the $gus^{mps}/+$ matings), it is much easier to test the efficacy of *in utero* therapies such as enzyme replacement, stem cell transplantation, and gene therapy on MPS VII fetuses. Because pathologic alterations in LSD are already present in newborn mice (7), the most effective treatment for LSD may require initiation of therapy *in utero*. This report provides clinical and

pathologic information that will be a useful benchmark for evaluation of such therapeutic strategies in the gus^{mps2J}/gus^{mps2J} mouse.

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