Syngeneic Bone Marrow Transplantation Reduces the Hearing Loss Associated With Murine Mucopolysaccharidosis Type VII

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MPS VII mice are deficient in β-glucuronidase and share many clinical, biochemical, and pathologic characteristics with human mucopolysaccharidosis type VII (MPS VII). We have shown that syngeneic bone marrow transplantation (BMT) prolongs survival and reduces lysosomal storage in many organs of the MPS VII mouse. In this report, we quantify the hearing loss and determine the impact of syngeneic BMT on the development of deafness and the associated pathology in the MPS VII mouse. Eleven weeks after syngeneic BMT performed at birth, treated MPS VII mice had normal auditory-evoked brainstem responses (ABR), whereas untreated MPS VII mice had ABR thresholds 43 dB higher than normal. Treated MPS VII mice had β-glucuronidase-positive cells in the temporal bone and in the subepithelial connective tissue of the external auditory canal. There was less thickening of the tympanic membrane and middle ear mucosa and decreased distortion of the ossicles and cochlear bone. Although transplanted MPS VII mice had increased ABR thresholds by 33 weeks of age, four of the six had thresholds 12 to 32 dB lower than untreated mutants. These data indicate that syngeneic BMT in newborn MPS VII mice prevents early hearing loss and, in some animals, results in long-term improved auditory function.

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Because the abnormal pathology in the ears and the hearing loss was prevented in MPS VII mice expressing a full-length human \( \beta \)-glucuronidase transgene, we determined the effectiveness of other forms of therapy for treatment of the hearing deficits and ear histopathology. Here we show that MPS VII mice have a severe to profound hearing loss at 5 to 15 weeks of age as evidenced by an auditory-evoked brainstem response (ABR) threshold that is 42 dB higher than normal animals. This hearing defect was not evident in 11-week-old MPS VII mice treated with BMT as newborns. Histochemical and histopathologic studies after BMT in MPS VII mice showed \( \beta \)-glucuronidase–positive cells in the temporal bone adjacent to the ear and in the subepithelial connective tissue of the external auditory canal, and less severe histologic alterations in the ear compared with untreated MPS VII mice. These data indicate that BMT performed early in life dramatically improves the clinical and pathologic findings in the ears of mice with MPS VII. From these studies, we also conclude that ABR thresholds and ear pathology provide useful objective criteria for evaluating the effectiveness of somatic cell gene transfer and enzyme replacement therapies for MPS VII.

### MATERIALS AND METHODS

**MPS VII mice and BMT.** Homozygous mutant (\( \text{gus}^{mp} \)/\( \text{gus}^{mp} \)) and phenotypically normal (+/?) mice were obtained from the B6.C-H-B2m\(^{-}\)/ByBi-\( \text{gus}^{mp} ?/+) mutant strain maintained by E.H.B. at The Jackson Laboratory. Phenotypically normal (+/?) littermates include +/+ and +/\( \text{gus}^{mp} \) mice. Heterozygous mice are completely normal by all criteria tested except for a 50% decrease in \( \beta \)-glucuronidase activity. Homozygous normal donors were obtained from a separate colony that is syngeneic except at the H-2 b locus. Animals for this study were either from a pedigreed colony, maintained by strict brother-sister matings, or from a nonpedigreed colony in which the offspring were never more than one generation from the inbred colony. Five normal (+/+) and seven mutant (\( \text{gus}^{mp} \)/\( \text{gus}^{mp} \)) mice at 5 to 15 weeks of age were tested for ABRs to determine the extent of hearing loss in both sexes.

Normal mice were tested for ABR thresholds to determine the effect of age on hearing. Six mice from 9.5 to 11 weeks, five mice from 34 to 38 weeks, and four mice from 56 to 69 weeks of age were tested for hearing.

On the day of birth, seven +/+ and six \( \text{gus}^{mp} \)/\( \text{gus}^{mp} \) mice were exposed to 200 rads delivered by a 137Cs irradiator and received an intravenous injection of 100 \( \mu \)L containing \( 1 \times 10^8 \) bone marrow (BM) cells from the femurs of adult female +/+ mice. Five untreated, age-matched \( \text{gus}^{mp} \)/\( \text{gus}^{mp} \) mice served as controls. The transplanted animals and untreated mutant controls were tested for ABR thresholds at 11 weeks of age and the transplanted animals were retested at 33 weeks of age. At 33 weeks of age, all of the untreated mutant controls had died.

**Hearing tests.** Mice were tested for hearing by means of the ABR as previously described. Each mouse was anesthetized with 0.2 to 0.5 mL of Avertin (tribromoethanol, 20 mg/mL) administered intraperitoneally. Needle electrodes were inserted subdermally on top of the head (active), below the left ear (reference), and on the dorsosacrum (ground). Broadband clicks (1 to \( \pm 16 \) kHz) or pure-tone pips (8, 16, or 32 kHz) were delivered via headphones adapted with funnels loosely coupled to the pinnae of the mouse. The ABR threshold was determined for each stimulus in descending intensities (10 dB) and finally at \( \pm 5 \) dB. Each determined threshold exhibited at least two of the four or five peaks of the ABR waveform within 1 to 5 milliseconds after onset of the stimulus. ABR thresholds of 40 to 80 dB indicate a moderate to severe hearing loss, whereas thresholds above 80 dB indicate a profound hearing loss. Statistical significance of the hearing results was determined using Student’s t-test.

The 12 untreated mice initially tested (five mutant and seven phenotypically normal) were stimulated with broadband clicks generated from a Bio-logics averager (Traveler Express E, Mundelien, IL) and the remaining mice were tested with broadband clicks and with pure-tone pips generated with the Intelligent Hearing System averager. The results from the latter instrument were corrected as per calibrated acoustic output at the tip of the funnels. Broadband clicks stimulate most of the hair-cells over approximately the lower half of the frequency range of the mouse cochlea; thus, clicks evoke, from a normally functioning ear, a characteristic ABR wave form at the lowest thresholds. By contrast, pure-tone pips stimulate relatively few hair cells, which are attuned to each frequency. Age-related, sensorineural hearing loss generally progresses (raised thresholds) from highest to lowest frequencies, accompanied by loss of hair-cells from the base to apex of the cochlea, respectively. Conductive hearing loss, involving ME function, generally impedes hearing across all pip frequencies and broadband clicks.

**\( \beta \)-Glucuronidase activity.** The relative levels of donor BM engraftment were determined at 33 weeks of age by measuring \( \beta \)-glucuronidase activity in homogenates of liver from the transplanted mutant animals. In mice that were perfusion fixed, a section of liver was removed before fixation. \( \beta \)-Glucuronidase activity was measured using a fluorometric assay with 4-methylumbelliferyl-\( \beta \)-D-glucuronide (Sigma Chemical Co, St Louis, MO) as substrate. Protein determinations were performed by the Coomassie dye binding assay. \( \beta \)-Glucuronidase specific activity was calculated as nmol 4-methylumbelliferyl/hydrolyzing protein and expressed as a percent of normal (+/+). Activity.

**Histochemistry.** Active \( \beta \)-glucuronidase was identified histochemically as previously described. Ear capsules were dissected from a 33-week-old treated MPS VII mouse, a treated normal mouse, and a 27-week-old untreated mutant. Ten-micron-thick cryosections from a 33-week-old treated MPS VII mouse, and four transplanted normal mice were examined by light microscopy. Included in this group were untreated MPS VII mice, and four transplanted normal mice were examined by light microscopy. Included in this group were untreated MPS VII mice.
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Normal mice have an average ABR threshold of 19 dB (±4.2) with a range of 15 to 25 dB. Untreated MPS VII mice have an average threshold of 61 dB (±8.0) with a range of 55 to 75 dB. The difference between the average ABR thresholds of normal and MPS VII mice is 42 dB.

ABR thresholds at broadband frequencies and 8, 16, and 32 kHz were compared in normal animals tested at 9.5 to 11, 34 to 38, and 56 to 69 weeks of age (Fig 2). As the mice age, there is a significant hearing loss at every frequency. Normal animals between 9.5 and 11 weeks of age have average ABR thresholds at broadband frequencies of 22.5 dB, which is similar to the normal mice described in Fig 1. At 34 to 38 weeks of age, the average ABR threshold increases significantly to 33 dB (P < 0.05) at the broadband frequencies. Corresponding increases in the average ABR threshold were also observed at the 8-, 16-, and 32-kHz frequencies. Mice older than 1 year (56 to 69 weeks), have ABR thresholds significantly higher (P < 0.01) than mice at 34 to 38 weeks of age. There was no consistent difference between the auditory-evoked thresholds of male or female animals.

There is a minor difference (statistically significant at P < 0.1) in ABR thresholds between the untreated mutant mice described in Figs 1 and 3. This apparent difference is most likely because of the different instruments used to test the mice because a similar difference is observed between the age-matched untreated normals presented in Figs 1 and 2.

**BMT corrects the hearing loss in MPS VII mice.** Figure 3 shows the ABR thresholds of untreated and BMT-treated MPS VII mice compared with BMT-treated normal mice at 11 weeks of age. Compared with treated normal animals, untreated MPS VII mice of identical age had elevated ABR thresholds at every frequency. The increase in the average ABR threshold was 46, 46, 39, and 41 dB at the broadband frequency, 8, 16, and 32 kHz, respectively. The ABR thresholds of treated MPS VII mice did not differ significantly from those of treated normal animals. Compared with untreated MPS VII mice, the average ABR thresholds of the treated MPS VII mice were decreased by 43, 41, 40, and 36 dB at the broadband and 8-, 16-, and 32-kHz frequencies, respectively (Fig 3). The average difference in ABR thresholds at all frequencies between the treated normals and the untreated mutants was 43 dB. Similarly, the average difference between the treated mutants and untreated mutants was 40 dB.

The same mice treated with BMT were retested at 33 weeks of age. All of the treated mutants were still alive and clinically well, whereas all five untreated mutants had died. The ability of BMT to increase survival beyond the average 21-week life span of untreated MPS VII mice is consistent with our previous findings. Compared with 11 weeks of age, both the treated normal mice and treated mutant mice had elevated ABR thresholds at 33 weeks (Fig 4). Treated normals had an average increase of 13 dB at the various frequencies that was similar in magnitude to the age-related increases in ABR thresholds in untreated normal animals.

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**Fig 1.** ABRs from individual normal mice (+/?;■) and mice with mucopolysaccharidosis type VII (MPS VII; ○) are represented as thresholds (dB) indicating the minimum intensity where an ABR waveform was attained. The mice were stimulated with broadband frequencies (clicks) ranging from 1 to ±16 kHz. The average ABR click thresholds are 19 ± 4.2 dB and 61 ± 8 dB for the normal and MPS VII mice, respectively. The average difference between the two groups is 42 dB.

**Fig 2.** The effect of age on ABR thresholds in normal mice of the same strain is shown at different stimulation frequencies. (■), Normal mice ranging in age from 9.5 to 11 weeks of age (n = 6); (●), mice from 34 to 38 weeks of age (n = 5); and (○), mice from 56 to 69 weeks of age (n = 4). The animals were stimulated with broadband clicks (1 to ±16 kHz), and 8-, 16-, and 32-kHz pure tone pips. The error bars represent ±1 SD.
(Fig 2). In contrast, the treated MPS VII mice had an average increase in ABR threshold that was greater than 13 dB. However, the range of ABR thresholds among the treated mutants was also larger. Two MPS VII mice were virtually deaf (90 to 100 dB) by 33 weeks of age, whereas another animal in this group had ABR thresholds comparable with those of the treated normal animals at every frequency. At the broadband frequencies, four of the six BMT-treated MPS VII mice had ABR thresholds that were 12 to 32 dB lower than 11-week-old untreated mutants.

**Biochemical and histochemical analysis.** β-Glucuronidase activity was detectable in the liver of all treated MPS VII mice at 33 weeks of age. β-Glucuronidase activity in liver homogenates ranged from 0.6% to 1.7% normal, which approximated the levels previously reported in MPS VII mice receiving 200 rads of conditioning radiation and $10^8$ syngeneic BM cells.  

Histochemical analysis of the ear from a 33-week-old treated MPS VII mouse identified β-glucuronidase positive cells (red) in the temporal BM and subepithelial connective tissue of the external auditory canal (Fig 5). The distribution of positive cells in the temporal BM is similar to that observed in a treated normal mouse of the same age; however, we estimate that the number of β-glucuronidase containing cells is only 1% to 5% of normal. No β-glucuronidase activity was observed in an untreated MPS VII animal.

**Improved pathology of the ear after BMT.** Histologically, the ears from the transplanted MPS VII mice showed a marked decrease in the pathologic alterations characteristic of murine MPS VII (Fig 6).  

In general, the tympanic membrane was thinner and there was less otitis in treated MPS VII mice compared with untreated MPS VII mice (Fig 6, A, B, and C). The ossicles (malleus [M] and incus [I]) still contained vacuolated cells but osteocyte alteration was less apparent, even in the oldest treated MPS VII animals, when compared with the same bones from untreated MPS VII mice (Fig 6, D, E, and F). However, the lysosomal storage material in chondrocytes appeared unchanged. ME mucosal thickening was present in a few of the transplanted MPS VII mice, but it was less severe and the large mucosal polyps present in untreated MPS VII mice were not observed. The cochlear bone from the treated MPS VII mouse shows marked improvement with reduction in the size of the perivascular spaces (Fig 6, G, H, and I). The external auditory canal (EC) of treated MPS VII mice still contained laminated cerumen in most cases.

**DISCUSSION**

The mucopolysaccharidoses are heritable disorders that affect many organ systems and cell types.  

Progressive conductive and sensorineural hearing loss are common clinical findings in the mucopolysaccharidoses, including MPS VII. Severe pathologic alterations have been observed in the ears of humans and mice with MPS VII.  

Here we show that β-glucuronidase-deficient mice have a severe to profound hearing deficit as early as 5 to 15 weeks of age.
The previously documented pathologic alterations are sufficient to explain a hearing loss with conductive and sensorineural components.\textsuperscript{15}

Unlike untreated MPS VII mice, MPS VII mice that received BMT at birth have ABR thresholds that remain essentially the same as those of normal mice receiving the same treatment until at least 11 weeks of age. Although the dramatic improvement in hearing correlates with an improvement in histopathology of the ear, it is not clear which histologic finding or findings contribute significantly to the physiologic improvement. The presence of $\beta$-glucuronidase–positive cells in the marrow spaces around the ear and rare positive cells in the subepithelial connective tissue of the external auditory canal may directly contribute sufficient enzyme early in life to prevent or slow progression of the otic pathology. It was previously shown that only a small amount of enzyme activity (1\% to 5\% of normal levels) is required to reverse existing disease in the liver and spleen of adult mice.\textsuperscript{19,20} It was also shown that low doses of conditioning radiation followed by BMT at birth reduced many of the bone abnormalities in MPS VII mice, despite the lack of histochemically demonstrable $\beta$-glucuronidase activity in osteocytes.\textsuperscript{17} Although the amount of $\beta$-glucuronidase in the serum of transplanted mice is below the level of detection of our assay, it is possible that minute amounts of circulating enzyme may also contribute to the improved histopathology. Recombinant $\beta$-glucuronidase delivered intravenously to newborn mice results in dramatic improvement in many tissues including the bones and brain.\textsuperscript{22}

The treated normal mice appeared to have a slight hearing deficit at 11 weeks of age compared with untreated normal mice. This deficit occurs before the age-related hearing loss and may be caused by the radiation-induced damage observed after BMT initiated at birth.\textsuperscript{13}

Hearing loss occurs with age in both treated MPS VII and treated normal mice. The hearing loss in the treated normal mice is similar in magnitude to that observed with aging in untreated normal mice of the same strain. Although age-related hearing loss is normally observed in several mouse strains, histologic correlates for the defects have not been identified.\textsuperscript{23}

The hearing loss by 33 weeks of age in some of the treated MPS VII animals was greater than that seen in age-matched normal controls. In fact, two of the treated MPS VII mice were deaf by this age. The otitis media and bony abnormalities characteristic of MPS VII were present, though less severe, than in untreated MPS VII animals. These abnormalities together with the external auditory canal cerumen may have contributed to the progressive hearing loss in some of the animals.

Although treated MPS VII mice had, on average, more hearing loss at 33 weeks of age than treated normal mice, the variation between individual animals was large. As mentioned above, two treated MPS VII mice were deaf by this age. The otitis media and bony abnormalities characteristic of MPS VII were present, though less severe, than in untreated MPS VII animals. These abnormalities together with the external auditory canal cerumen may have contributed to the progressive hearing loss in some of the animals.

Why the treated MPS VII mice differ so greatly in response to BMT is unclear. The individual differences in ABR thresholds do not correlate with the level of donor BM engraftment. The treated MPS VII mouse that had the lowest ABR threshold at 33 weeks of age actually had the lowest level of engraftment as determined by liver $\beta$-glucuronidase activity and a mouse that had profound hearing loss had approximately twice the enzyme activity. The variation may reflect individual differences in the severity of otitis media. Possibly, administration of antibiotics to treat the otitis media and clearing the cerumen from the external auditory canal...
could have slowed the progression of the hearing loss and generated a more uniform response to BMT therapy.

Previous studies showed the efficacy of BMT as a means to reduce lysosomal storage in many tissues of mice with MPS VII. However, with the exception of the increase in longevity, it has been difficult to quantify the effect of BMT on any clinical or physiologic deficits associated with murine MPS VII. For example, we were unable to evaluate the effect of early BMT on the behavioral defects in the MPS VII mouse because the conditioning radiation induced abnormalities in the normal mice receiving the same treatment. By contrast, the present study clearly shows that BMT at birth has a profound effect in preserving auditory function early in life.

BMT has been proposed as a means of therapy for human patients with MPS I, II, IV, and VII. Children with MPS I who received BMT at an early age have persistent levels of α-L-iduronidase, a decrease in intracranial hypertension, and slowed progression of their mental retardation. In these disorders for which BMT has been proposed, conductive and sensorineural hearing loss is an important clinical finding. A recent study of 12 children with MPS I who were treated with BMT showed that 8 of the children had improved auditory function to the point that they did not require amplification devices, whereas 4 showed no improvement. Our data on the MPS VII mice clearly show a slowed progression of the hearing deficits, β-glucuronidase–positive cells in and around the ear, and an improvement in otic histopathology. There is good correlation between the response seen in children with MPS I and mice with MPS VII which provides cause for optimism regarding the improvement in hearing by BMT for lysosomal storage diseases. Finally, regular assessment of ABR thresholds provides another parameter to evaluate the effectiveness of other novel therapies for these disorders such as gene therapy and enzyme replacement.

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