Targeted Disruption of the Acid α-Glucosidase Gene in Mice Causes an Illness with Critical Features of Both Infantile and Adult Human Glycogen Storage Disease Type II*

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We have used gene targeting to create a mouse model of glycogen storage disease type II, a disease in which distinct clinical phenotypes present at different ages. As in the severe human infantile disease (Pompe Syndrome), mice homozygous for disruption of the acid α-glucosidase gene (6^neo^/6^neo^) lack enzyme activity and begin to accumulate glycogen in cardiac and skeletal muscle lysosomes by 3 weeks of age, with a progressive increase thereafter. By 3.5 weeks of age, these mice have markedly reduced mobility and strength. They grow normally, however, reach adulthood, remain fertile, and, as in the human adult disease, older mice accumulate glycogen in the diaphragm. By 8–9 months of age animals develop obvious muscle wasting and a weak, waddling gait. This model, therefore, recapitulates critical features of both the infantile and the adult forms of the disease at a pace suitable for the evaluation of enzyme or gene replacement. In contrast, in a second model, mutant mice with deletion of exon 6 (Δ6/Δ6), like the recently published acid α-glucosidase knockout with disruption of exon 13 (Bijvoet, A. G., van de Kamp, E. H., Kroos, M., Ding, J. H., Yang, B. Z., Visser, P., Bakker, C. E., Verbeet, M. P., Oostra, B. A., Reuser, A. J. J., and van der Ploeg, A. T. (1998) Hum. Mol. Genet. 7, 53–62), have unimpaired strength and mobility (up to 6.5 months of age) despite indistinguishable biochemical and pathological changes. The genetic background of the mouse strains appears to contribute to the differences among the three models.

In glycogen storage disease type II (GSDII), 1 an autosomal recessive disorder, the failure of acid α-glucosidase (GAA, acid maltase, EC 3.2.1.20) to hydrolyze lysosomal glycogen leads to the abnormal accumulation of large lysosomes filled with glycogen in certain tissues (2). The most severe form, Pompe Syndrome, is a rapidly progressive disease in which heart failure is fatal in infancy. In milder forms, there is progressive skeletal muscle weakness, and death may result from pulmonary failure secondary to diaphragmatic weakness as late as the seventh decade.

There is currently no effective therapy, but several candidate therapies, based on the discovery that acid α-glucosidase, like many other lysosomal enzymes, is secreted and can be taken up through cell surface mannose-6-phosphate receptors on other cells (3–5), are already under development (6–12). These studies stimulated efforts to create a mouse model suitable for testing enzyme replacement and gene therapies. Bijvoet et al. (1) recently reported the generation of knockout mice which develop generalized glycogen storage and cardiomyogal but remain phenotypically normal.

We describe here the generation of two models: 1) knockout mice in which the GAA gene is disrupted by a neo insertion in exon 6 (6^neo^/6^neo^) and 2) mutant mice in which exon 6 of the GAA gene and the neo gene are removed by Cre/lox-mediated recombination (Δ6/Δ6) (13). In both models, animals develop biochemical and pathological changes similar to those in humans, but only 6^neo^/6^neo^ mice show early signs of reduced mobility and muscle strength. By 8–9 months of age 6^neo^/6^neo^ mice develop a weak, waddling gait, and progressive muscle wasting.

EXPERIMENTAL PROCEDURES

Construction of Targeting Vector, Transfection of Embryonic Stem (ES) Cells, and Breeding—GAA genomic clones were isolated from a 129/Sv mouse genomic library. A plasmid containing both the neomycin-resistance (neo) gene and the herpes virus thymidine kinase gene in the pBluescript vector (a gift of Dr. R. Froio) served as the backbone of the targeting vector (14). The organization of the targeting construct is shown in Fig. 1A. A genomic fragment extending from an XhoI site in intron 2 to a BamHI site in exon 6 was inserted into the XhoI site between the thymidine kinase and neo genes. In addition, a termination codon and a new EcoRV site were introduced within exon 6 upstream from the neo gene. Next, a genomic fragment containing the remainder of exon 6 and exons 7 through 13 was cloned into the SalI site downstream of the neo gene. Two loxp sites were inserted into introns 5 and 6. The resulting vector has ~2.7 kb of homology upstream and ~4.3 kb of homology downstream of the neo gene. The linearized vector was electroporated into 129/Sv/RW4 ES cells (Genome Systems Inc.), and the resulting neo-positive (G418-resistant), thymidine kinase-negative (ganciclovir-resistant) clones were screened by Southern analysis. Chimeric mice were generated by blastocyst injection of heterozygous ES cells into 3.5-day C57BL/6 embryos. Six independent cell lines containing the disrupted GAA allele were used to make chimeras that were bred to C57BL/6 females to generate heterozygous mice (F_1). Four mutant lines were then established through germ line transmission;
heterozygous F₁ mice derived from two independent cell lines, 2-55 and 2-86, were intercrossed to mice homozygous for the disrupted allele (F₂ and F₃). Alternatively, F₁/2-55 and F₁/2-86 heterozygous mice were bred to EIIa-cre transgenic mice (FVB/N) for Cre-mediated deletion (Δ6/Δ6) of the neo gene and exon 6 of the GAA gene in vivo.

Enzyme Assay and Western Blot Analysis—GAA activity in the homogenates of skeletal muscle, liver, heart, and tail was measured as conversion of the substrate 4-methylumbelliferyl-α-D-glucoside to the fluorescent product umbelliferone as described previously (1, 15). Tissue pellets were homogenized in lysis buffer (300 mM NaCl, 50 mM Tris, 2 mM EDTA, 0.5% Triton X-100) with proteinase inhibitors (4 mM Pefabloc SC, 10 μg/ml aprotinin, 10 μg/ml leupeptin). Samples (50 μg protein) were electrophoresed on 10% SDS-polyacrylamide gel electrophoresis gels and electrotransferred to nitrocellulose membranes. The blots were blocked with bovine serum albumin and incubated with rabbit antiserum to human placental GAA or rabbit antiserum to human urine GAA (kindly provided by Dr. F. Martiniuk and Dr. A. J. J. Reuser). Immunodetection was performed with goat anti-rabbit IgG conjugated to horseradish peroxidase in combination with chemiluminescence (ECL, Amersham Life Science Inc.).

Isolation of RNA and DNA, cDNA Synthesis, RT-PCR, and Southern Analysis—RNA was isolated from skeletal muscle and liver using a Total RNA Kit (Qiagen). First strand cDNA synthesis was primed from 0.5 μg total RNA with 50 ng of random hexamers according to the manufacturer's instructions (Boehringer Mannheim). Two μl of the cDNA sample were used as a template for PCR amplification with primers flanking the neo gene: cctttactggcgagggcag (exon 5 sense) and ggacaatggcggtcgaggagta (exon 7 antisense) or taccacctggagggagac (exon 4 sense) and cggccatcctggtgcagctcccgca (exon 8 antisense). The second set of primers was used to detect any possible transcripts in which the neo gene may be spliced out. PCR reactions were carried out for 35 cycles that consisted of 50-s denaturation at 95 °C, 50-s annealing at 55 °C, and 2-min extension at 72 °C using PCR SuperMix (Life Technologies, Inc.). Genomic DNA isolated from ES cells or mouse tails was digested with EcoRV, electrophoresed on 1% agarose gels, and transferred to Nytran membranes. The hybridization probe was generated by PCR, and labeled by the random hexamer method after gel purification.

Histology—For electron microscopy, tissues were fixed in phosphate-buffered saline containing 4% formaldehyde and 2% glutaraldehyde followed by post-fixation in 1% osmium in 0.1 M cacodylate buffer. The tissues were rinsed in an aqueous solution containing 4.5% sucrose, dehydrated in a series of graded alcohol solutions, rinsed in 100% propylene oxide, and embedded in epoxy resin. Thin sections (60 to 70 nm) were double-stained with uranyl acetate and lead citrate. The stained sections were stabilized by carbon evaporation and photographed with a Hitachi H7000 electron microscope operated at 75 kV.

**Table I**

| Origin of the mice used for behavioral testing | Cell line | Mouse line |
|-----------------------------------------------|-----------|------------|
| 2-55/cre                                      | 2-55       | 2-55/cre   |
| 3/0/0                                         | 3/0/0      | 3/0/0      |
| 2-86/cre                                     | 2-86/cre   | 2-86/cre   |

* F₁ mice/F₂ mice/F₃ mice (n).

**Fig. 1.** Disruption of the GAA gene in mouse ES cells, germ line transmission of the disrupted allele, and expression of murine GAA in wild-type and Δ6/Δ6** mice. A, structure of the targeting vector (middle) and a partial restriction map of the GAA locus before (upper) and after (lower) homologous recombination. A neo cassette was inserted in exon 6 of the gene. A 300-bp probe in intron 2 (external to the targeting construct) detects a ~6-kb fragment from wild-type DNA and a mutant specific fragment of ~2.7 kb. Restriction sites are: B, BamHI; RI, EcoRI; RV, EcoRV; H, HindIII; X, XbaI. B, initial screen of the targeted GAA clones by Southern blot hybridization analysis (left). The EcoRV 2.7-kb fragments indicated the expected recombinant allele. Southern blot analysis of tail DNA from wild-type (+/+), heterozygous (+/-), and homozygous (-/-) offspring in the F₁ generation derived by crossing F₁ carriers for the GAA disruption (right). C, RT-PCR analysis of muscle cDNA from wild-type (+/+), heterozygous (+/-), and homozygous (-/-) mice. The primers in exons 5 and 7 flank the neo gene detected a 265-bp amplification product in the wild-type (+/+) and heterozygous (+/-) but not in homozygous (-/-) mice (top). Similarly, the primers in exons 4 and 8 detect a 478-bp product only in the wild-type (+/+) and heterozygous (+/-) mice (bottom). The RT-PCR negative control (NC) was carried out by omitting RNA from reverse-transcription reaction. M, DNA marker. D, Western analysis (shown for liver). The blot was probed with rabbit IgG to human urine GAA (I) or human placental GAA (II).
For light microscopy, sections from tissues were fixed in 10% formalin, processed, embedded in paraffin, and stained with hematoxylin-eosin or periodic acid-Schiff (PAS) by standard methods.

**Behavioral Testing**—All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Locomotor activity in an open field was measured in a Digiscan apparatus (model RXYZCM, Omnitech Electronics). Total distance, horizontal activity, and vertical activity were measured by the total number of photocell beam breaks in 10- or 15-min intervals over 1 h, and data averaged over these periods were used for analysis. Three to six independent testing sessions were conducted for each group over a period of 1–2 weeks. Male mice were tested at ages 3.5–6, 8–9, and 10.5–22 weeks. Fourteen 6 neo/+, 11 6 neo/6 neo, 8 6∆/+, and 9 6Δ/6 mice were used for the test. The origin of the mice which were phenotypically tested is indicated in Table I. Statistical analyses were performed using the one-way analysis of variance test (Sigmastat program). The ability to hang upside down from a wire screen placed 60 cm above a large housing cage was measured as latency to fall into the cage.

**RESULTS**

The murine GAA gene was disrupted by insertion of neo into exon 6, with the expectation that the disruption would completely block gene expression (6 neo/6 neo). In addition, loxP sites were placed in the introns flanking the disrupted exon 6 so that exon 6 could be precisely removed (Δ6) by mating to Cre-producing mice. In humans, a similar splicing mutation around exon 6 is associated with a relatively mild phenotype (16).

**Generation of Mice with Exon 6 Disruption of the GAA Gene**—By homologous recombination in ES cells, we created a mutant GAA allele in which a neo cassette disrupts the gene within exon 6 (Fig. 1A). A termination codon and a new EcoRV site were introduced into exon 6 upstream from the neo gene. Translational termination at the stop codon in exon 6 would result in the synthesis of a truncated protein of ~36 kDa. The frequency of recombination was 1 in 4 G418/ganciclovir-resistant clones. Recombinant clones were used to produce chimeric mice. Heterozygous mice (F1) derived from two independent cell lines (2-55 and 2-86) carrying the targeted allele were used for further breeding. Genotyping of the mice generated by intercrossing of heterozygotes (Fig. 1B) revealed the expected Mendelian ratio, indicating no effect on embryonic development.

Reverse transcription-PCR with two sets of primers flanking the neo gene detected wild-type products in the wild-type (+/+) and heterozygous (+/−) but not in 6 neo/6 neo (−/−) mice (Fig. 1C), indicating that normal mRNA is not made in homozygotes. However, mRNA amplification with primers in exon 12 (sense) and exon 14 (antisense) downstream from the neo gene detected a low level of transcripts in 6 neo/6 neo mice (not shown). Similarly, RT-PCR with primers in exon 5 (sense) and the neo gene (antisense) detected a very low abundance message in the 6 neo/6 neo; reamplification of the PCR product with nested primers followed by sequencing established that the termination codon introduced into exon 6 upstream from the neo gene remained intact (not shown).

In homozygous mice, no GAA protein was detected by Western analysis (Fig. 1D) using antibodies against either human urine or human placental GAA. The absence of functional protein in 6 neo/6 neo mice was confirmed by enzyme assay in the lysates of multiple tissues (Table II). The residual levels of enzyme activity (at the standard pH 4.3) in the muscle, heart, and tail samples of 6 neo/6 neo mice did not exceed the background level found in a fibroblast cell line from an infantile patient (0.64 nmol of 4-methylumbelliferyl-α-D-glucoside/h/mg of protein; cell line 4912) in which mRNA is not expressed (17). At low pH 3.6 (1) the enzyme activity was below the detection limits (0.7–1.0 ng of 4-methylumbelliferalone/10-μl reaction).

**6 neo/6 neo Mice Accumulate Glycogen in Lysosomes—Abnormal lysosomal glycogen storage was found in the heart and skeletal muscle of 6 neo/6 neo mice. Electron microscopy showed the progressive accumulation of membrane-limited organelles within the lysosomes (not shown). Over time, the lysosomes increased in size and number (Fig. 2, b–d and a–c). Importantly, in the 6 neo/6 neo mice, there is a significant reduction in the number of myofi-
brils, loss of lateral myofibrillar registration, and signs of sarcomere degradation, especially the deformation at the Z lines. Some lysosomes appear broken, suggesting that the leakage of lysosomal proteases may have contributed to the damage of the muscle structure.

Light microscopy (at 8 weeks) showed PAS-positive, diastase-sensitive material in vacuoles in the heart and skeletal muscle (Fig. 3, b and d). In animals examined at 14 weeks, the diaphragm showed PAS-positive vacuoles by light microscopy (Fig. 3, f).

**6neo/6neo Mice Display Significant Behavioral Abnormalities**—Although the mutant mice appeared normal, when placed in an open field environment 6neo/6neo mice consistently performed significantly worse than heterozygous littermates by several measures of locomotion (Fig. 4). Reduced activity was registered as early as 3.5 weeks of age and was particularly striking for vertical motion (Fig. 4, bottom panel). Similarly, in the wire-hang task, which measures muscular function and grip strength, 6neo/+ mice outperformed 6neo/6neo littermates. At 15–16 weeks of age, 6neo/6neo mice were almost never able to hold on to the inverted screen for more than 2 min (once in 12 tests), whereas in 8 of 12 tests 6neo/+ littermates were able to remain hanging for more than 2 min, and 4 of 12 heterozygous littermates were still holding on at 5 min when the test was stopped. Older mice (8–9 months of age) show obvious signs of muscle weakness with a weak, waddling gait and muscle wasting (Fig. 5). Offspring from independent mutant mouse lines were phenotypically indistinguishable.

**Generation and Characterization of Mice with an In-frame Deletion of Exon 6 of the GAA Gene (Δ6/Δ6)**—In the second model, the disrupted exon 6 of the GAA gene and the neo gene were totally excised from early embryos by breeding 6neo/+ mice (F1/2-55 and F1/2-86; 129/C57BL/6 background) to transgenic homozygous EIIa-cre (18) mice (FVB/N background) in which the adenovirus promoter confines the expression of Cre to an early stage of pre-implantation development. F1 heterozygous (mouse lines 2-55/cre and 2-86/cre) for exon 6-deleted allele were subsequently intercrossed to obtain F2 and F3 homozygous mice (Δ6/Δ6). Cre-mediated deletion was detected by PCR with primers in exon 5 and exon 7 (Fig. 6). As expected, the genomic sequence in homozygous mice contained the 5′ part of intron 5, then a single loxP site in place of exon 6, followed by the 3′ part of intron 7 and exon 7 of the gene (not shown). RT-PCR with two sets of primers (in exons 5/7 and exons 4/8) showed that the mutant mRNA is produced (Fig. 7A), and that in this mRNA exon 5 is spliced to exon 7, resulting in a precise in-frame deletion of exon 6 (not shown).

The Δ6/Δ6 mice were similar to the 6neo/6neo animals with respect to the level of enzyme activity measured in tail skin, muscle, and liver (not shown), absence of protein (Fig. 7B), and accumulation of lysosomal glycogen in skeletal muscle, heart, and diaphragm (Fig. 8). Strikingly, however, unlike the 6neo/6neo mice, their performance in the open field was similar to that of heterozygous Δ6/+ littermates derived from two mouse lines (Fig. 9, Table I). Interestingly, in all measures of activity, the Δ6/+ mice outperformed the 6neo/+ animals, indicating a
genetic difference between the two mouse strains. So far (up to 6.5 months of age) the Δ6/Δ6 mice have not developed any clinical symptoms.

**DISCUSSION**

We have used an efficient method for generating two allelic mutations at the murine GAA locus. The approach required the production of only one targeted mouse line with an exon 6 disrupted allele, which served as a progenitor of the second line with exon 6 deleted allele. Since the targeted locus contains two 
loxP sites flanking exon 6, the removal of the exon was performed simply by mating to transgenic mice carrying Cre recombinase. The two models were designed to replicate a range of clinical phenotypes: 6neo/6neo mice for a severe phenotype, and Δ6/Δ6 mice for a milder disease. A milder phenotype was predicted in the Δ6/Δ6 mice since a similar, though not identical defect in a patient, splicing out exon 6 and the inclusion of 7 new amino acids encoded by 21 nucleotides from IVS6, resulted in 5–7% of residual enzyme activity and a juvenile form of the illness (16). Both mouse models, however, resulted in apparently complete “knockout,” as shown by the virtual absence of enzyme activity and the absence of GAA protein.

In humans, the severity and the age of onset of GSDII appear to depend largely on the level of residual activity of the enzyme. Lack of enzyme activity or extremely low levels (≤1–2%) are associated with a fatal infantile cardiomyopathy, whereas levels of 10–20% are associated with an adult onset indolent skeletal myopathy (19–21). Unlike humans, recently described knockout mice (9 months old) (1) and the Δ6/Δ6 mutants (6.5 months old) described here do not show clinical signs despite a severe enzyme deficiency.

In contrast, 6neo/6neo mice develop a progressive muscle weakness detectable as early as 3.5 weeks of age. The pathologic findings in 6neo/6neo mice indicate accumulation of lysosomal glycogen in the skeletal muscle and diaphragm, as in the adult human disease, and an even greater accumulation in heart, a hallmark of infantile disease. Tests of cardiac function will allow determination of the effects of the glycogen accumulation in the heart. In quantitative tests of mobility and strength, 6neo/6neo mice moved less, especially in the vertical direction, and could not hold on to a wire screen nearly as long as 6neo/+ littermates. By 8–9 months, clinical signs of muscle weakness and muscle wasting are obvious. Longer observation will be necessary to determine if this reduced strength affects lifespan and if glycogen accumulation in the diaphragm reduces lung function.

Thus, the 6neo/6neo model has features of both the adult and the infantile forms of the human disease, but the effects are attenuated. This difference in severity and in pace between mice and humans is not surprising since the factors which promote lysosomal glycogen storage are largely obscure. In humans, for example, the deposition of glycogen is very different from tissue to tissue within the same patient and from patient to patient or even sibling to sibling although they may bear the same mutation(s).
Of related interest in that regard are the observations that although both the $6^{neo}/6^{neo}$ and $D^6/D^6$ mice have negligible enzyme activity and accumulate glycogen in skeletal and heart muscles, the $6^{neo}/6^{neo}$ are weak in open field and wire hang testing, but the $D^6/D^6$ are not. The phenotypic difference between the two models described here cannot be explained by the presence of a $neo$ gene in the targeted locus of the $6^{neo}/6^{neo}$ mice: in both the phenotypically affected $6^{neo}/6^{neo}$ model and a recently published phenotypically normal model with insertion of a $neo$ gene in exon 13, a hybrid GAA-$neo$ mRNA was detected by RT-PCR. Furthermore, we have studied the expression of the $neo$ gene in a mouse strain with disruption of the HexA gene which is known to perform normally in the open field (14). In this strain, abundant $neo$ transcripts were detected in both liver and muscle by RT-PCR and sequencing (not shown), thus further indicating no effect of $neo$ phosphotransferase on mobility and muscle strength.

It is possible that the accumulation of glycogen is different in the muscles crucial for the activities tested; or that accumulation of glycogen in other sites such as the nervous system differs in the two models; or that weakness is related not only to the amount of accumulated glycogen. Indeed, the structural changes in myofibrillar structure may relate to other factors besides simple glycogen accumulation which are involved in

**FIG. 6. Detection of Cre-mediated deletion ($\Delta 6/\Delta 6$).** A, the structure of the targeted GAA locus before (upper) and after (lower) in vivo Cre-mediated deletion. The two arrowheads represent the loxP sites. B, the primers in exon 5 (sense) and exon 7 (antisense) detect 687- and 505-bp PCR fragments corresponding to the wildtype and exon 6 deleted alleles, respectively (left panel). Genotype analysis of the offspring from a $6^{neo}/+ \times EIIa-cre$ cross (right panel). PCR analysis of tail DNA in the F$_2$ (top) and F$_3$ (bottom) generations. Both the 687- and 505-bp bands were detected in heterozygotes (+/−), only the larger band is seen in the wildtype (+/+), and only the smaller band in homozygous (−/−) mice. M, DNA marker; NC, negative control.

**FIG. 7. Expression of the exon 6-deleted allele.** A, RT-PCR analysis of muscle cDNA from $\Delta 6/\Delta 6$ mice. Primers in exons 4 and 8 detect a 354-bp amplification product; primers in exons 5 and 7 detect a 150-bp product. The sizes of the products correspond to those expected for mRNA with exon 6 deleted. Each PCR was done in duplicate. The RT-PCR negative control (NC) was carried out by omitting RNA from reverse-transcription reaction in which both sets of primers were used. M, DNA marker. B, Western analysis (shown for liver). The blot was probed with rabbit IgG to human placental GAA. Lane 1, $6^{neo}/+$; lane 2, $6^{neo}/6^{neo}$; lane 3, $D^6/D^6$.

**FIG. 8. Analysis by light microscopy of skeletal muscle, heart, and diaphragm in $\Delta 6/\Delta 6$ mice.** PAS-stained sections of a, skeletal muscle at 8 weeks of age (× 750); b, heart at 8 weeks of age (× 750); and c, diaphragm at 11 weeks of age (× 1000).
out mice (22, 23). As shown in Fig. 9, the background activity of the Cre strain control mice is substantially greater than that of the controls for the Δ6/Δ6neo mice, suggesting that other genes influence behavior in the tests, and only in the less active strain is the additional insult of glycogen accumulation reflected in poorer performance. Such strain differences may account for the apparent absence of weakness in the recently published model (1), which, like the Δ6/Δ6 model described here, was created on the 129/FVB background.

It should be noted that the level of residual activity in the exon 13 model was somewhat higher (2.6% in muscle and 3.8% in heart) than the levels in the Δ6/Δ6neo and the Δ6ΔΔ6 mice when measured at the same low pH 3.6. At that low pH, neither of the knockout strains described here had detectable activity in tail skin, muscle, or heart. It is possible that a residual low level of enzyme activity contributes to rescue of the phenotype of the exon 13 published knockout (1). Longer observation of the Δ6Δ6 mice (oldest animals are 6.5 months of age) may clarify this point.

Although all of the models created so far could be used for testing proposed gene therapy or enzyme replacement since the pathological and biochemical changes closely resemble those in humans, the nondestructive and easily testable phenotypic abnormalities of the Δ6/Δ6neo model suggest that it would be the preferable choice.

REFERENCES

1. Bijvoet, A. G., van de Kemp, E. H., Kroos, M., Ding, J. H., Yang, B. Z., Visser, P., Bakker, C. E., Verbeek, M. P., Oostra, B. A., Reuser, A. J. J., van der Ploeg, A. T. (1998) Hum Mol Genet 7, 53–62
2. Hirschhorn, R. (1995) In The Metabolic and Molecular Basis of Inherited Disease (Scrivner, C. R., Beaudet, A. L., Sly, W. S., and Valle, D. eds) pp. 2443–2464, McGraw-Hill, New York
3. van der Ploeg, A. T., Loonen, M. C., Bolhuis, P. A., Busch, H. M., Reuser, A. J. J., and Galjaard, H. (1988) Pediatr. Res. 24, 90–94
4. van der Ploeg, A. T., Kroos, M. A., Willemsen, R., Bruns, N. H., and Reuser, A. J. (1991) J Clin Invest. 87, 513–518
5. Neufeld, E. F. (1991) Annu. Rev. Biochem. 60, 257–280
6. van der Ploeg, A. T., Bolhuis, P. A., Wolfert, R. A., Visser, J. W., Loonen, M. C., Busch, H. F., and Reuser, A. J. (1988) J Neurol. 235, 392–396
7. van Hove, J. L., van der Ploeg, A. T., Busch, H. F., Reuser, A. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 89, 65–70
8. Zaretskaya, J. Z., Candotti, F., Boerkoel, C. F., Adams, E. M., Yewdell, J. W., Blase, R. M., and Plotz, P. H. (1997) Hum. Gene Ther. 8, 1559–1563
9. Kessler, P. D., Potsakoff, G. M., Chen, X., McQuiston, S. A., Colosi, P. C., Matelis, L. A., Kurtzman, G. J., and Byrne, B. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14082–14087
10. Bijvoet, A. G., Kroos, M. A., Pieper, F. R., de Boer, H. A., Reuser, A. J., van der Ploeg, A. T., and Verbeek, M. P. (1996) Biochim. Biophys. Acta 1308, 93–96
11. Pauly, D. F., Johns, D. C., Matelis, L. A., Lawrence, J. H., Byrne, B. J., and Reuser, A. J. J., Ploos van Amstel, H. K., Ausems, M. G., and Verbeet, M. P., Oostra, B. A., Reuser, A. J. J., van der Ploeg, A. T., and Verbeet, M. P. (1996) Biochim. Biophys. Acta 1308, 93–96
12. Martiniuk, F., Mehler, M., Pellicer, A., Tzall, S., La Badie, G., Hobart, C., Ellenbogen, A., and Hirschorn, R. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9641–9644
13. Mathen, A., Yamanaka, S., Johnson, M. D., Grinberg, A., Westphal, H., Crawley, J. N., Tantrik, M., Suzuki, K., and Proia, R. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9975–9979
14. Hermans, M. M., Kroos, M. A., van Beuningen, J., Oostra, B. A., and Reuser, A. J. (1991) J Biol. Chem. 266, 13507–13512
15. Adams, E. M., Becker, J. A., Griffith, L., Segal, A., Plotz, P. H., and Raben, N. (1997) Hum Mutat. 10, 128–134
16. Adams, E. M., Becker, J. A., Griffith, L., Segal, A., Plotz, P. H., and Raben, N. (1997) Hum Mutat. 10, 128–134
17. Martinuk, F., Mehler, M., Pellicer, A., Tzall, S., La Badie, G., Hobart, C., Ellenbogen, A., and Hirschorn, R. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9641–9644
18. Lasko, M., Pichel, J. G., Gorman, J. R., Sauer, B., Okamoto, Y., Lee, E., Alt, F. W., and Westphal, H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5860–5865
19. Reuser, A. J. J., Kroos, M. A., Hermans, M. M., Bijvoet, A. G., Verbeek, M. P., van Diggelen, O. P., Kleijer, W. J., and van der Ploeg, A. T. (1995) Muscle Nerve 18, 836–842
20. Kroos, M. A., Van der Kraan, M., van Diggelen, O. P., Kleijer, W. J., Reuser, A. J. J., Van den Boogaard, M. J., Ausems, M. G., Ploos van Amstel, H. K., Poenaru, L., Nienlo, M., and Wevers, R. (1995) J. Clin. Invest. 95, 836–837
21. Raben, N., Nichols, R. C., Boerkoel, C., and Plotz, P. (1995) Muscle Nerve 3, S70–S74
22. Erickson, R. P. (1996) Bioessays 18, 993–998
23. Wilson, J. M. (1996) J. Clin. Invest. 97, 1139–1144

Fig. 9. Locomotor activity of mice with exon 6 deletion (heterozygotes, Δ6+/; homozygotes, Δ6ΔΔ6), and exon 6 disruption (heterozygotes, 6neo+/; homozygotes, 6neoΔ6neo). Top panel, mean (±S.E.) horizontal activity per min in the open field (measured by the number of photocell beam breaks). Middle panel, mean (±S.E.) total distance (cm) per min in the open field. Bottom panel, mean (±S.E.) vertical activity per min in the open field (measured by the number of photocell beam breaks). Each bar represents the performance of 8–14 animals, and ~200 intervals (10 min each) were averaged for each bar.