Neuromuscular degeneration \( (nmd) \): a mutation on mouse Chromosome 19 that causes motor neuron degeneration

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Abstract. Neuromuscular degeneration, \( nmd \), is a spontaneous autosomal recessive mutation in the mouse producing progressive hindlimb impairment caused by spinal muscular atrophy. We used an intersubspecific intercross between B6.BKs-\( nmd^{2J/+} \) and \( Mus\) \( musculus\) \( castaneus\) (CAST/Ei) to map the \( nmd \) mutation to mouse Chromosome (Chr) 19 with the most likely gene order: \( nmd^{2J}-\) (D19S62, Pygm)-Cnfn-Pomc2-D19Mit16-Cyp2c-Go1. \( nmd \) maps near muscle deficient, \( mdf \), and has a very similar clinical phenotype, but allele tests and histological differences suggest that \( nmd \) is a distinct mutation at a different locus. Although closely linked, \( nmd \) recombined with the candidate genes muscle glycogen phosphorylase, \( Pygm \), and ciliary neurotrophic factor, \( Cnfn \).

Introduction

Human motor neuron diseases constitute a wide variety of devastating syndromes characterized by progressive debilitating muscular atrophy secondary to irreversible neuronal degeneration. Recent success has been made in genetically mapping amyotrophic lateral sclerosis type 1 (ALS1) to human Chr 21q22 (Siddique et al. 1991), ALS2 to 2q31-35 (Hentati et al. 1992), and one form of spinal muscular atrophy (SMA) to 5q12.2-13 (Wirth et al. 1993). Research with protective and restorative agents is also progressing. Human motor neuron diseases constitute a wide variety of devastating syndromes characterized by progressive debilitating muscular atrophy secondary to irreversible neuronal degeneration.

Materials and methods

Mouse strains and crosses. All mice used in these studies were reared under modified barrier conditions in The Jackson Laboratory Mouse Mutant Resource (Davison 1990). The \( nmd \) mutation has occurred twice at The Jackson Laboratory (TJL). The first mutant allele, \( nmd \), occurred in the CBA/J inbred strain in the early 1980s. The second occurrence, \( nmd^{2J} \), was discovered in the C57BL/Ks-m db (misty, diabetes) colony by Pat Burch in 1987. DNA from mutants and sibling controls of the CBA/J-nmd/+ strain was preserved in TJL DNA Resource; however, embryos were not cryopreserved. The CBA/J-nmd/+ strain was discarded when replaced by the \( nmd^{2J} \) remutation. The studies in this paper describe the \( nmd^{2J} \) allele.

Histological techniques. For histological preparation, 20 \( nmd^{2J}/nmd^{2J} \) and 10 +/+ littermate controls ranging in age from 2 to 4 weeks were deeply anesthetized with tribromoethanol (Avertin) and fixed by intracardiac perfusion of Bouin’s solution following a flush with physiological saline. After demineralization in Bouin’s, multiple cross sections of all portions of the spinal cord and hind brain were prepared. Cross and longitudinal sections of lumbar muscles and fore- and hindlimbs were also sampled. For light microscopy, sections were stained with hematoxylin and eosin (H&E) or with luxol fast blue-cresyl echt violet (LFB-CV). For ultrastructural studies, two \( nmd^{2J}/nmd^{2J} \) mutants at 3 weeks of age were fixed by intracardiac perfusion of dilute Karnovsky’s solution. Samples of lumbar spinal cord were dissected and processed by standard methods for electron microscopy.

Mapping protocols. To map the chromosomal location of \( nmd \) we mated a female \( M.\) \( m.\) \( castaneus\) (CAST/Ei) to a heterozygous (\( nmd^{2J/+} \)) male from the strain B6.BKs-\( nmd^{2J/+} \). F2 progeny were intercrossed, and only F2 homozygous mutants contributed to the mapping data. The isoenzyme locus glutamate oxalate transaminase, \( G01 \), was assayed from kidney extracts according to the cellulose acetate method of De Lorenzo and Ruddle (1970). Genomic DNA samples were prepared by standard SDS/proteinase K lysis, phenol/chloroform extraction, and ethanol precipitation. DNA was digested with \( Taq1 \) and analyzed by the Southern blotting protocol of Johnson and associates (1992). The probes for pro-opiomelanocortin-beta, \( Pomc2 \), and cytochrome P450 subfamily 1c, \( Cyp2c \), were purchased from the American Type Culture Collection. The probe for ciliary neurotrophic factor, \( Cnfn \), was kindly provided by J. Roder and J. Henderson of Mr. Sinai (1989). The probe for muscle glycogen phosphorylase, \( Pygm \), was donated by M. Cerasa of York University. Ontario and T. Glasser of Brigham and Women’s Hospital, Boston (Glasser et al. 1989). The probe for \( D19S62 \) was provided by Michael Seldin of Duke University, Durham, North Carolina. \( D19Mit16 \) was typed as a dinucleotide repeat polymorphism by PCR amplification, modified from Dietrich and colleagues (1992) with primer pairs from Research Genetics.
Results

Phenotypic description and genetic characterization. Homozygous \textit{nmd}^{2J}/\textit{nmd}^{2J} mutants are easily classified visually by 2 weeks of age by their dorsally contracted hind legs and impaired movement (Fig. 1). They cannot extend their legs to stand erect or assume full posture on all four limbs. They clench their hindlimbs when picked up by the tail and they are unable to grasp a cage cover when held against it. They crawl forward by relying on their forelimbs to pull them along while their hindlimbs struggle to push against the surface. They can maintain proper balance, however, and do not circle, bob their heads, or fall over sideways. They are capable of righting themselves when intentionally inverted. Analysis progress, and the deteriorating mutants rarely survive past 3½ weeks of age. Occasionally, however, some will live for several months when left with their parents and when normal siblings have been discarded from the litter. Because most homozygous mutants die before breeding age, the mutation is currently maintained by progeny tests for heterozygous pairs. We are backcrossing \textit{mnd}^{2s} to C57BL/6J so that after 8–10 backcross generations colony maintenance by transplantation of \textit{mnd}^{2s}/\textit{mnd}^{2s} ova ries to a histocompatible host will be possible. Since heterozygotes appear visually and histologically normal and one-fourth of their progeny are affected mutants, the \textit{mnd}^{2J} mutation is inherited as a recessive allele.

Histopathology. Pathologic changes in \textit{mnd}^{2J}/\textit{mnd}^{2J} were observed in skeletal muscle and in motor neurons of the spinal cord and sympathetic nervous system. Many skeletal muscles revealed groups of tiny muscle fibers intermixed with normal-sized fibers (Fig. 2a). The severity of the lesions varied depending on location. Sections of proximal forelimb and hindlimb musculature had focal areas of severe atrophy, while sections of distal musculature of both fore- and hindlimb had generalized severe muscle atrophy with replacement by adipose tissue. Sections of paraxial lumbar muscle had few affected muscle groups. Cross-sections through the musculature of the head had groups of atrophic muscle fibers in the masseter and temporalis muscles. The distribution of affected fibers in the right and left muscles was not symmetrical. These findings are consistent with random denervation of muscles with consequent neurogenic atrophy.

The nuclei and perikarya of affected alpha motor neurons were pale staining in hematoxylin and eosin (H&E) and in luxol fast blue-cresylecht violet (LFB-CV) sections, although the outlines of affected cells were delineated by each stain. We have designated these affected neurons “fading neurons” (Fig. 2b). This alteration in staining affinity is not an artefact, as shown by the normal staining of adjacent neurons. Moreover, in our studies of many other mouse neurological mutants, we have never observed this type of cell-specific change. In no case did we observe any cellular reactive changes around the fading neurons, such as inflammatory cell infiltrations or proliferation of astrocytes, microgliocytes, or satellite cells. Motor neurons affected are primarily in the lumbar region. Serial cross-sections of lumbar spinal cord of three 21-day-old \textit{mnd}^{2J}/\textit{mnd}^{2J} mice revealed on average one affected neuron per four cross sections. In older mutants, all sections of lumbar spinal cord showed affected neurons. Motor neurons of the cervical and thoracic regions of the spinal cord and cranial motor nuclei rarely showed degenerating neurons. Control siblings had no anomalies. Neurons in various stages of degeneration were seen in each mutant examined. We observed fading neurons in the sympathetic chain in two serially sectioned spinal cords, but have not yet studied sympathetic end organs in sufficient detail to determine whether such degenerative changes have any associated lesions in visceral organs. Ultrastructurally, fading neurons were not swollen and were of normal shape. They were pale because cytoplasmic and nuclear organelles were less densely packed than in normal neurons, but organelles present—mitochondria, Golgi, endoplasmic reticulum—appeared normal (Figs. 2c–e).

Genetic linkage analysis. An intersubspecific intercross with CAST/Ei was chosen because this wild-derived and now inbred strain produces highly heterozygous and fertile F_{1} hybrids with standard inbred strains (Johnson et al. 1992). Because both parents were \textit{F}_{1} hybrids and only homozygous mutants were analyzed, each progeny genotype represents the product of two informative meioses. The most probable gene order, \textit{mnd}–(1D19Sel2, Pygm)–\textit{Cntf-Pomc2-D19Mit16-Cyp2c-Got1}, was determined with Mapmaker version 1.9 (Lander et al. 1987). This order, as shown in Fig. 3, is approximately 100 times more probable than the second best order of (1D19Sel2, Pygm)–\textit{mnd}–\textit{Cntf-Pomc2-D19Mit16-Cyp2c-Got1}. The interlocus genetic distances of the \textit{mnd} map were calculated from Map Manager version 2.5 (Manly 1993). Individual mouse genotypes at all loci on Chr 19 typed in this cross have been deposited as a Map Manager file in the Mouse Genome Database, Accession Number MGD-CREX-162.

Comparison of our data with the composite genetic map of the mouse (GBASE 1994) shows that \textit{mnd} maps about 4 cm proximal to muscle deficient, \textit{mdf}. However, the map position of \textit{mdf} is not as precise (Sweet 1983). We tested these two independent mutations for alleleism to see whether they are mutations of the same gene. Fourteen 3 to 5-month-old \textit{F}_{1} progeny from an allele test mating of a \textit{mdf/mdf} female of the strain B6C3Fe-a/a \textit{mdf} (Womack et al. 1980) to a \textit{mnd}^{d1+} male showed no visible or histological neuropathy or myopathy. A second independent alleleism test between \textit{mdf}+ (one each sex) matted to \textit{mnd}^{d1+} (one each sex) yielded no affected animals in a total of 52 offspring. Normal litter sizes of 7–10 pups per litter suggested there was no prenatal lethality. Although background differences may inhibit mutant expression, false alleleism tests are rare. Hence, \textit{mnd} and \textit{mdf} appear to be distinct genes.

Fig. 1. Homozygous 3½ week \textit{mnd}^{2J}/\textit{mnd}^{2J} male on the right with littermate + control on the left. Note that overall atrophy increases along the length of the body towards the lumbar region.