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Chromosomal Mapping of the Peroneal Muscular Atrophy (pma) Gene in the Mouse

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Abstract: *We conducted chromosomal mapping of the* pma *gene that is a causative gene in the peroneal muscular atrophy mouse, which shows a club foot at birth and unusual gait due to a dropped foot in the adult. Linkage analyses using backcross progeny revealed a significant linkage between the* pma *gene and three microsatellite markers,* D5Mit263 *at 73 cM,* D5Mit141 *and* D5Mit97 *at 74 cM on Chr 5. The gene order was determined as follows: centromere-*D5Mit263*-[2.65 cM]-*D5Mit141*-[2.56 cM]-*pma*-[5.13 cM]-*D5Mit97*-telomere.*

Key words: *Chr 5, mouse, peroneal muscular atrophy*

Esaki *et al.* [3] reported an autosomal recessive mutant showing an anomaly of the hind limb found in the process of inbreeding of a line derived from CF1 outbred stock. The major phenotype of this mutant is a club foot at birth and unusual gait due to a dropped foot in the adult as shown in Fig. 1A and 1B. Anatomical and pathological studies have revealed the absence of common peroneal nerves, which leads to atrophy of the posterior crural muscles (Fig. 1D) compared with normal muscles (Fig. 1C). This strain was tentatively named, PMA, which means a phenotype of peroneal muscular atrophy and the mutation was named *pma* [3]. Unfortunately, detailed genetic studies of this anomaly have not been performed to date. We report chromosomal mapping of the *pma* gene in this paper.

The MSM/Ms strain used for mating experiments was introduced from the National Institute of Genetics, Mishima, Japan. F_1 and backcross progeny were obtained by mating between PMA-*pma*/*pma* and MSM/Ms-+/+. Genotypes of the *pma* gene were determined by observation of morphology of the hind foot and the common peroneal nerve. Linkage between the *pma* gene and markers on autosomes, except for sex chromosomes, was studied using backcross progeny. Fifty-six markers on Chr 1 to Chr 19 showing genetic polymorphisms between PMA and MSM were selected for the linkage study of the *pma* gene as follows: *D1Mit1* (8.7), *D1Mit7* (41) and *Pep3** (71) on Chr 1, *D2Mit12* (50.3) on Chr 2, *Car2** (10.5), *D3Mit6* (23.3), *D3Mit17* (71.8) and *D3Mit19* (87.6) on Chr 3, *D4Mit81* (38), *D4Mit76* (55.7), *D4Mit54* (66) and *D4Mit13* (71) on Chr 4, *D5Mit13* (20), *D5Mit10* (54), *D5Mit68* (65),

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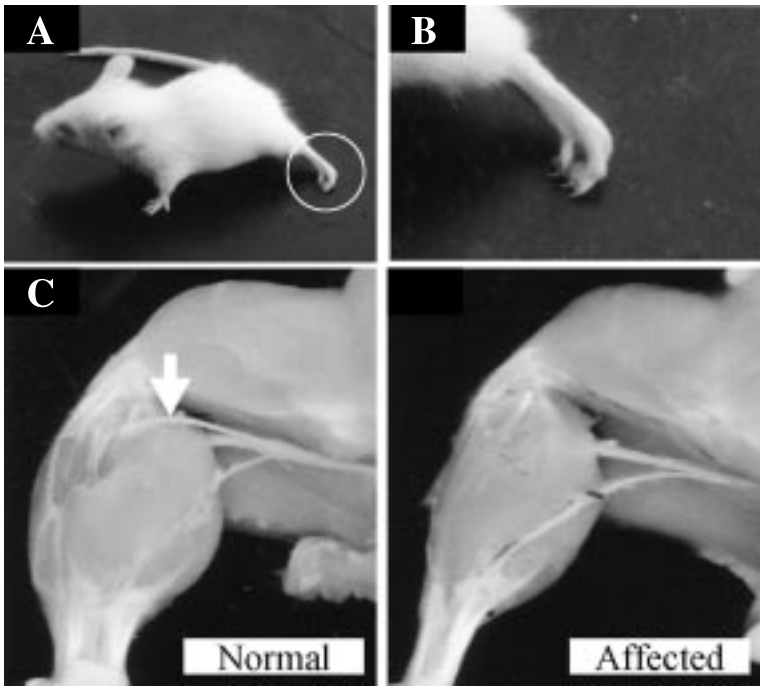

Fig. 1. Morphological characteristics of the PMA (peroneal muscular atrophy) mouse. A: A PMA mouse with peroneal muscular atrophy in hind limbs, B: Clubfoot caused by absence of the common peroneal nerve, C: Developed common peroneal nerve (shown by white arrow) in the normal mouse, and D: Undeveloped common peroneal nerve in the abnormal mouse.

D5Mit29 (72), *D5Mit30* (72), *D5Mit263* (73), *D5Mit141* (74), *D5Mit97* (74), *D5Mit32* (78), *D5Mit167* (78), *D5Mit43* (83) and *D5Mit101* (81) on Chr 5, *D6Mit10* (48.7) on Chr 6, *D7Mit57* (4) and *Hbb** (50) on Chr 7, *D8Mit3* (10), *Es1** (43), *Es2** (52) and *D8Mit14* (67) on Chr 8, *D9Mit2* (17) and *Mod1* (48) on Chr 9, *D10Mit28* (4), *D10Mit1* (6), *D10Mit16* (16), *D10Mit3* (21), *D10Mit66* (49), *D10Mit68* (51.5), *D10Mit70* (59) and *D10Mit35* (69) on Chr 10, *D11Mit100* (68) and *Es3** (74) on Chr 11, *D12Mit37* (1) on Chr 12, *D13Mit26* (38) on Chr 13, *D14Mit10* (3), *D14Mit18* (16.5), *D14Mit30* (29) and *Gnrh* (39.5) on Chr 14, *D15Mit29* (42.8) and *D15Mit16* (61.7) on Chr 15, *D16Mit9* (4) on Chr 16, *D17Mit76* (54.6) on Chr 17, *D18Mit40* (37) on Chr 18, and *D19Mit29* (4) and *D19Mit1* (52) on Chr 19. Markers on each chromosome are described in centimorgans (cM) according to their order from the centromere to telomere based on Mouse Genome Informatics [5]. Biochemical markers with asterisks (*) were detected by CAME (cellulose acetate membrane electrophoresis). Microsatellite DNA markers, as simple sequence length polymorphisms (SSLPs), were amplified by PCR followed by agarose gel electrophoresis to determine genotypes of the markers. Primer sets for microsatellite DNA markers were purchased from Research Genetics, Inc. (Invitrogen, CA, USA).

Mating experiments with PMA-*pma*/*pma* and MSM/Ms-+/+ to demonstrate inheritance of peroneal muscular atrophy were carried out (Table 1). Fourteen (5 females and 9 males) F_1 mice were obtained by mating female PMA-*pma*/*pma* mice and male MSM-+/+ mice. Their hind limbs were morphologically normal. Reciprocal crosses between F_1-*pma*/+ and PMA-*pma*/*pma* were performed to produce backcross progeny. Seventy-six out of 115 mice (72 from crosses of F_1-*pma*/+ and PMA-*pma*/*pma* and 43 from crosses of PMA-*pma*/*pma* and F_1-*pma*/+) were normal and 39 were abnormal. Segregation ratios of normal and abnormal mice in both crosses were the same. Abnormal mice with unilateral peroneal muscular atrophy (five right atrophy and five left atrophy) were observed at a frequency of 15.4%. Abnormal

Table 1. Mating experiments performed to demonstrate inheritance of peroneal muscular atrophy

Crosses	Total Offspring	Phenotypes of Offspring		Ratio of Normal and Abnormal
		Normal (Female: Male)	Abnormal (Female: Male)	
PMA-*pma*/*pma* × MSM–+/+ (F_1)	14	14 (5: 9)	0	1.0 : 0
F_1-*pma*/+ × PMA–*pma*/*pma* (BC)	72	47 (20: 27)	25 (16: 9)	1.9 : 1
PMA-*pma*/*pma* × F_1–*pma*/+ (BC)	43	29 (13: 16)	14 (8: 6)	2.1 : 1

BC: backcross.

mice with unusual gait caused by a dropped foot showed consistently absence of the common peroneal nerve(s). If the peroneal muscular atrophy is controlled by a single autosomal recessive gene, the ratio of normal to abnormal mice should be 1: 1 according to Mendelian inheritance. However, the ratio obtained in this study was 2: 1. There is a possibility that peroneal muscular atrophy may be controlled by two autosomal recessive genes, one a major gene (*pma*) and the other a minor (or a modifier) gene. Esaki *et al.* reported that the penetrance and expressivity of the gene were complete in the original or related strains, but incomplete in the other genetic backgrounds [3]. In our study, the same phenomena were observed using the MSM/Ms strain derived from the Japanese wild mouse (*M. m. molossinus*). However, we could not determine whether the two genes hypothesis or the penetrance hypothesis is valid.

Linkage analyses were carried out to determine the chromosome on which the *pma* gene is located. One hundred and twelve BC progeny contributed to this analysis, since genotyping of three markers were unsuccessfully performed on three individuals of the 115 BC progeny. A significant linkage was observed between the *pma* gene and three microsatellite markers (*D5Mit263* at 73 cM [7], *D5Mit141* [6] and *D5Mit97* [8] at 74 cM) on Chr 5. Using a haplotype analysis method as shown in Fig. 2, the most likely gene order was determined as follows: centromere-*D5Mit263*-[2.65 cM]-*D5Mit141*-[2.56 cM]-*pma*-[5.13 cM]-*D5Mit97*. These recombination values were calculated using only the affected mice due to unreasonable higher recombination values in the normal mice. The positions of both *D5Mit141* and *D5Mit97* on Chr 5 have been reported as 74 cM in the MGI Map position [4]. However,

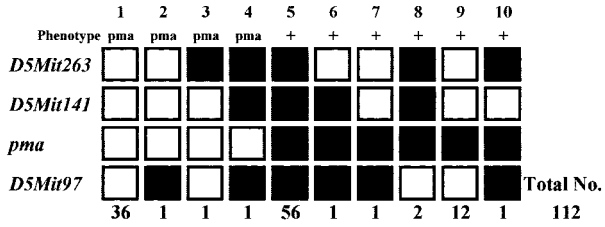

Fig. 2. Haplotype analyses carried out to demonstrate gene order of the *pma* gene and the microsatellite markers on Chr 5. Symbols: □ homozygous for the PMA allele at the *pma* and the marker loci; and ■ heterozygous for the PMA allele and the MSM allele.

we actually observed recombinants between them. Since the real distance between *D5Mit141* and *D5Mit97* is about 3 Mbp according to the MGSCv3 Sequence Map, recombination occurring between these two markers was reasonably understood. Mutations showing phenotypes similar to those of the *pma* mutation have not been reported on Chr 5 in the mouse.

Ashby *et al.* studied muscles that have never been exposed to motor or sensory neurons using PMA mice and demonstrated that primary myotubes develop in aneural muscles but require innervation for postnatal maintenance, while secondary myotube development is not dependent upon innervation [1]. Also, they showed that the development of secondary myotubes depends on neurally evoked electrical activity in primary myotubes [2]. As described by Nonaka *et al.* [9], the PMA mouse may be an experimental model for the study of arthrogryposis multiplex congenital or clubfoot deformity and may lead to understanding the muscle and nerve interaction during development, because the anomalous condition is present at birth with no progression.

Acknowledgments

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