SHORT COMMUNICATION

Conserved Linkage of Early Growth Response 4, Annexin 4, and Transforming Growth Factor α on Mouse Chromosome 6

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The mouse genes encoding early growth response 4 (*Egr4*), annexin IV (*Anx4*), and transforming growth factor α (*Tgfa*) have been mapped to a linkage group on mouse chromosome 6 that is conserved on human chromosome 2p11–p13. The genes are closely linked, with 0/215 recombinants between *Anx4* and *Tgfa* and 1/215 recombinants between these genes and *Egr4*. The genes are located approximately 2 cM distal to *mnd2*, a mouse mutation causing neuromuscular disease. The results demonstrate that *mnd2* is located at an internal position within this conserved linkage group.

Loci on mouse chromosome (Chr) 6 have been mapped to linkage groups on human chromosomes 2, 3, 7, 10, and 12 (6, 14). The conserved linkage group on human Chr 2p11–p13 includes the genes *Igk*, *Ly-2* (*Cd8a*), *Ly-3* (*Cd8b*), *Fabpl*, and *Sftp-3* (6, 14). The current study was undertaken to extend this linkage group by mapping the mouse homologs of three genes on human Chr 2p13: early growth response 4 (*EGR4*), annexin IV (*ANX4*), and transforming growth factor α (*TGFA*) (4, 15, 16). These loci were also tested as candidates for the *mnd2* mutation, which produces neuromuscular disease with muscle wasting and regression of spleen and thymus (9).

Egr4 encodes a zinc-finger transcription factor that is induced by nerve growth factor and by brain seizures (3, 4, 8). The human *EGR4* cDNA was cloned from a peripheral blood T lymphocyte cDNA library (13), and the homologous rat cDNA (NGFI-C) was cloned from pheochromocytoma PC12 cells (3).

Annexin IV is a member of the lipocortin family of calcium-dependent phospholipid-binding proteins. The annexin IV protein was isolated from human placenta based on its anticoagulant activity, but its *in vivo* function is uncertain (15).

Transforming growth factor α polypeptide mediates reversible transformation of cells *in vitro*. The polypeptide exhibits sequence homology with epidermal growth factor, with which it competes for receptor binding (11, 16). *Tgfa* was recently demonstrated to be allelic to the waved-1 locus on mouse Chr 6 (11, 12).

Egr4, *Anx4*, and *Tgfa* were mapped by Southern blot analysis on a (C57BL/6J-*mnd2* × CAST/Ei)F_2 mapping panel composed of mice homozygous for the *mnd2* mutation (9). The following hybridization probes were employed: a 2.1-kb rat *Egr4* cDNA clone pJDM450 (3), an 850-bp *Eco*RI–*Cla*I fragment from the human *ANX4* cDNA clone pPAP-II-B6 (15), and a 2.0-kb *Eco*RI–*Sal*I fragment from the rat *Tgfa* cDNA (10). Restriction fragment length polymorphisms were identified after digestion of genomic DNA from strains C57BL/6J and CAST/Ei with eight restriction endonucleases. The following CAST/Ei restriction fragments were used to follow segregation of these loci: an 8.6-kb *Eco*RI fragment

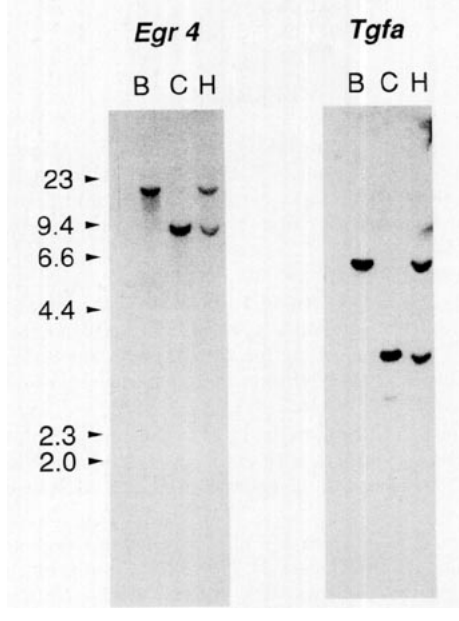

FIG. 1. Genetic variation of *Egr4* and *Tgfa*. Genomic DNA was analyzed by Southern blotting as previously described (1). The positions of bacteriophage λ *Hin*dIII fragments are indicated in kb at the left. B, C57BL/6J; C, CAST/Ei; H, heterozygote.

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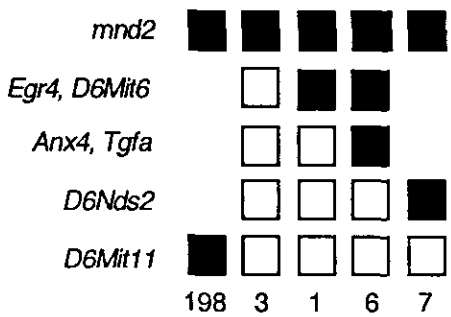

FIG. 2. Haplotype data for loci on Chr 6. Each column represents one observed haplotype from the (C57BL/6J-*mnd2* × CAST/Ei)F_2 mapping panel. The number of mice with each haplotype is indicated at the bottom. Haplotypes were inferred by assuming the absence of double crossovers. Solid squares, C57BL/6J alleles; open squares, CAST/Ei alleles. Primers for *D6Mit6* and *D6Mit11* (5) were obtained from Research Genetics (Huntsville, AL). PCR analysis was conducted as previously described (1).

hybridizing with the *Egr4* probe (Fig. 1), a 3.2-kb *Sac*I fragment hybridizing with the *Tgfa* probe (Fig. 1), and 1.4-kb *Taq*I and 2.0-kb *Sac*I fragments hybridizing with the *Anx4* probe (not shown).

Linkage analysis was conducted by tierred mapping. Two hundred fifteen homozygous *mnd2/mnd2* F_2 progeny were typed for the microsatellite *D6Mit11* (5). Recombinant animals were then typed for the microsatellite *D6Nds2* (2). Individuals with recombination between *mnd2* and *D6Nds2* were typed for *Egr4*, *Anx4*, and *Tgfa*.

Haplotypes from the mapping panel are presented in Fig. 2. The indicated gene order is (centromere)–*mnd2*–(1.4 ± 0.8)–*Egr4*, *D6Mit6*–(0.5 ± 0.5)–*Anx4*, *Tgfa*–(2.8 ± 2.8)–*D6Nds2*–(3.3 ± 1.2)–*D6Mit11*. *Anx4* and *Tgfa* did not recombine in 215 meioses, indicating that the loci are very closely linked (0.0 ± 1.4 cM, 95% confidence level). Gene order and relative positions are illustrated in Fig. 3.

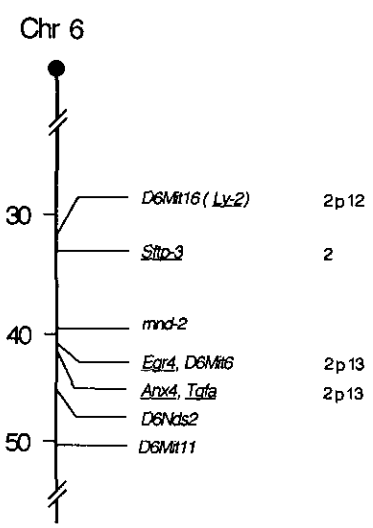

FIG. 3. Gene order on Chr 6. Map positions are indicated in centimorgans from the centromere. This map combines current data with previous data for *Ly-2* and *Sftp-3*, which were typed on a subset of the same mapping panel (9). The cytogenetic locations of human genes homologous to the underlined mouse loci are indicated at the right.

Our data are consistent with the recent assignment of *Tgfa* to the Chr 6 linkage group (7) and extend the previous data by the addition of the closely linked loci *Egr4* and *Anx4*. *EGR4*, *ANX4*, and *TGFA* were mapped cytogenetically to human Chr 2p13, but no information is available on gene order for the human loci. Linkage analysis in the mouse demonstrates that the three genes are separated by less than 1 cM (approximately 2 Mb). Conservation of gene order predicts that *EGR4* is proximal to the other two genes on human Chr 2. The data also demonstrate that *mnd2* is located at an internal position within this conserved linkage group. The most likely position for the human homolog of *mnd2* is therefore Chr 2p13.

The expression of the transcription factor gene *Egr4* in the nervous system and in lymphocytes made it an attractive candidate for the *mnd2* mutation, which produces defects in both of these tissues. However, the observed recombination (3/215) demonstrates that mutation of *Egr4* is not responsible for the *mnd2* phenotype.

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