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Localization of the human Chromosome 5q genes *Gabra-1*, *Gabrg-2*, *Il-4*, *Il-5*, and *Irf-1* on mouse Chromosome 11

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The γ_2 subunit of the $GABA_A$ receptor (*Gabrg-2*) and the interferon regulatory factor 1 (*Irf-1*) genes have been mapped for the first time on mouse Chromosome (Chr) 11. Their map position reinforces the observed synteny homology between Chr 11 and human Chr 5q. We also confirm the localization of the genes for the α_1 subunit of the $GABA_A$ receptor (*Gabra-1*) and interleukins 4 and 5 (*Il-4* and *Il-5*), as well as two anonymous DNA markers (*D11Mit1* and *D11Mit5*) on Chr 11.

Loci were mapped by use of a well-characterized intersubspecific backcross [(DF/B-*df*/*df* × *M. castaneus*) × DF/B-*df*/*df*] (Buckwalter et al. 1991; Karolyi et al. 1992). Interlocus distances obtained by typing this cross correspond well with other intersubspecific crosses (Buchberg et al. 1991). Progeny were genotyped at *Gabra-1, Gabrg-2, Irf-1,* and *Il-5* with restriction fragment length polymorphisms (RFLPs) (Fig. 1). Genotyping of *Il-4, D11Mit1,* and *D11Mit5* was performed by polymerase chain reaction (PCR) of sequences containing microsatellites (Fig. 2). Locus order is assumed to be that which results in the minimum number of recombination events.

The $GABA_A$ receptor is a chloride channel which binds γ-amino butyric acid (GABA). GABA is the major inhibitory neurotransmitter in the brain, as well as the site of action of benzodiazepams, barbiturates, alcohol, and many anticonvulsant and antipsychotic drugs (Zorumski and Isenberg 1991). The exact subunit composition of the receptor is unknown, but the γ_2 subunit contains a benzodiazepam binding site. We observed no recombination between *Gabrg-2, Gabra-1,* and *Adra-1* (Fig. 3). The placement of this cluster in our multipoint cross does not significantly differ from the previous localization of *Gabra-1* 9.9 ± 3.1 cM distal to *Rel* and 3.3 ± 2.3 cM proximal to *Il-3* (Keir et al. 1991). The human genes, *GABRA1* and *GABRG2,* have been localized on human Chr 5q34-q35 (Buckle et al. 1989) and on the same yeast artificial chromosome (YAC) clone (Wilcox et al. 1992; Warrington et al. 1992). Thus, the close linkage of *Gabrg-2* and *Gabra-1*

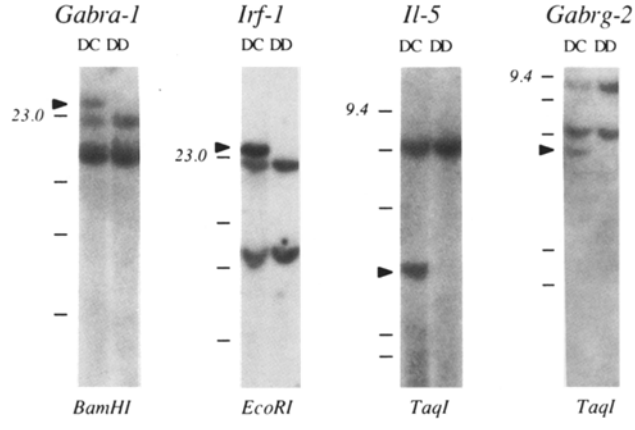

Fig. 1. RFLPs used to genotype backcross progeny at *Gabra-1, Irf-1, Il-5,* and *Gabrg-2.* Genomic DNA was digested with the indicated restriction enzyme. Southern blots and hybridizations were done essentially as described (Buckwalter et al. 1991). **Arrowheads** signify bands present in DNA from (DF/B-*df*/*df* × *M. castaneus*) F_1 mice (DC), but absent in homozygous DF/B-*df*/*df* (DD) mice. The positions of molecular weight markers, a *Hin*dIII digest of bacteriophage λ, are indicated at the left in kb. The *Gabra-1* probe was a full-length mouse cDNA kindly donated by W. Keir (University of Colorado) as a 2.7 kb *Eco*RI fragment. Plasmid pUC28-8, containing the mouse cDNA for *Irf-1* as a 2.1 kb *Eco*RI fragment was a gift of H. Harada (Osaka University). Mouse interleukin 5 cDNA (pmIL5-4G) was purchased from the ATCC. *Gabrg-2* was mapped with a 1.6 kb *Bam*HI-*Hin*dIII fragment of bovine cDNA from plasmid pBRγ2, provided by R. MacDonald (University of Michigan) and E. Barnard (Cambridge). Ninety-six progeny were typed for *Gabra-1* and 110 for *Gabrg-2. Irf-1* and *Il-5* were typed solely in the appropriate recombinant animals.

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in the mouse parallels the close linkage of these genes in the human.

No recombination was observed between interferon regulatory factor (*Irf-1*), interleukins 3, 4, and 5 (*Il-3, Il-4,* and *Il-5*), and granulocyte-macrophage colony stimulating factor (*Csfgm*). This cluster was localized 7.8 cM distal to the cluster *Adra-1, Gabrg-2,* and *Gabra-1*. Irf-1 is a transcriptional activator of interferon α and β and interferon-inducible genes (Fujita et al. 1989; Harada et al. 1990). *IRF-1* has been localized to human Chr 5q23-q31 (Itoh et al. 1991). Interleukins 3, 4, and 5 and Csfgm make up a family of cytokines with both hematopoietic growth factor and lymphokine activity (reviewed in Paul 1991). Because these genes have similar exon structure and are tightly clustered in both humans and mouse, it has been hypothesized that they arose by gene duplication. Irf-1 has no structural similarity to these cytokines, but it is coexpressed in some of the same cell types. In the mouse, *Il-3* and *Csfgm* are 14 kb apart (Barlow et al. 1987), and *Il-4* and *Il-5* are separated by 110 to 180 kb (Lee et al. 1989). The two gene pairs have not yet been

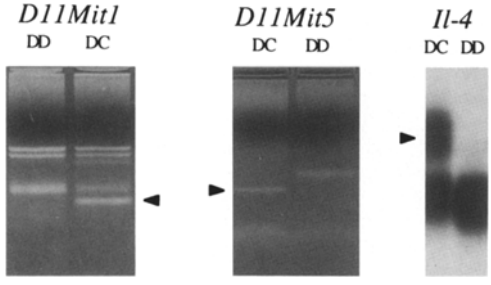

Fig. 2. Genotyping of the microsatellites *Il-4, D11Mit1,* and *D11Mit5*. PCR products were used to genotype backcross progeny at *D11Mit1, D11Mit5,* and *Il-4*. Genomic DNA (500 ng) was amplified with the primer sequences 5′-GGGTCTCTGAAGGCTTTGTG-3′ and 5′-TGAATACAGAAGCCACGGTG-3′ for *D11Mit1* and 5′-TTCTGTGAGCCTGGAGGAGT-3′ and 5′-TACAGGACTAGTT-TCCATTTGGG-3′ for *D11Mit5* (Dietrich et al. 1992). PCR reactions were carried out in a 25-μl volume with 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.001% (w/v) gelatin, 0.2 mM of dATP, dCTP, dGTP, and dTTP (Pharmacia), 0.5 μM each primer (University of Michigan DNA Facility and Research Genetics, Huntsville, Ala.), and 1–2 units of *Taq* polymerase. After initial denaturation at 94°C for 3 min, samples were amplified during 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, followed by incubation at 72°C for 10 min. The amplification products were separated by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining. The *M. castaneus*-specific bands, signified by the **arrowheads,** were approximately 110 bp for *D11Mit1* and 144 bp for *D11Mit5*. One hundred seventeen progeny were typed for *D11Mit1* and *D11Mit5*. *Il-4* was amplified from 100 ng of genomic DNA with the primers 5′-GTCTGCTGTGGCATATTCTG-3′ and 5′-GGCATTTCTCATTCAGATTC-3′ (Love et al. 1990). The PCR reaction was the same as for *D11Mit1* and *D11Mit5* except that 100 ng genomic DNA was used in a reaction volume of 10 μl and the dCTP concentration was 0.22 mM; 0.05 mM in cold dCTP (Pharmacia), and 0.17 μM (5 μCi) in α^{32}P-dCTP (Amersham). Primers were purchased from the University of Michigan DNA Facility. Samples were amplified during 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 30 s, followed by incubation at 72°C for 10 min. Denatured amplification products were separated by electrophoresis on 6% acrylamide/10 M urea gels and visualized by autoradiography. *Il-4* was typed solely in the appropriate recombinant animals.

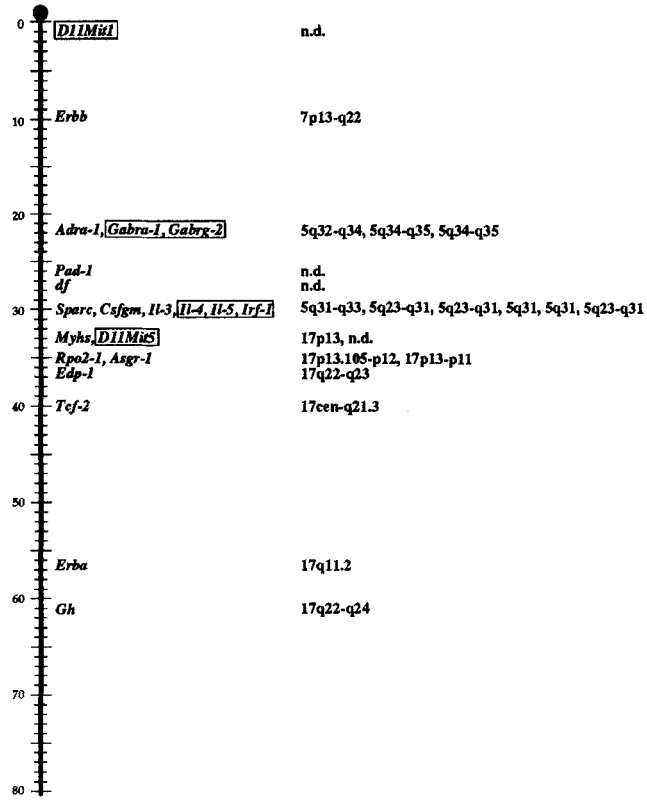

Fig. 3. Map of Chr 11. All of the loci shown have been mapped on the same backcross [(DF/B-*df*/*df* × *Casa*/*Rk*) × DF/B-*df*/*df*]. The loci described in this report are **boxed.** Human gene localizations, where known, are given on the **right** or indicated by **n.d.** if not determined (Kondo and Shimizu 1983; Yang-Feng et al. 1990; Buckle et al. 1989; Wilcox et al. 1992; Warrington et al. 1992; Swaroop et al. 1988; Le Beau et al. 1986, 1987; Sutherland et al. 1988a, 1988b; Itoh et al. 1991; Edwards et al. 1985; vanTuinen and Ledbetter 1987; Sanford et al. 1991; Wolf et al. 1992; Bach et al. 1990; Mitelman et al. 1986; Harper et al. 1982).

physically linked. Analysis of recombinant inbred lines has suggested that *Il-4* and *Il-5* may be proximal to *Il-3* (D'Eustachio et al. 1988; Wilson et al. 1990). The lack of recombinants between *Csfgm, Il-3, Il-4,* and *Il-5* in our linkage analysis confirms the close proximity of these genes in mice. Physical mapping will probably be necessary to determine the order of the cytokine genes and *Irf-1*.

Microsatellite markers are useful because they can be typed with PCR-based assays and because they are often polymorphic between standard inbred mouse strains. Thus, the inclusion of these markers in every multipoint analysis should facilitate comparisons of genetic distances between crosses. Toward that end, we have mapped *D11Mit1* and *D11Mit5* on our cross. We observed a distance of 29.4 cM between *D11Mit1* and *Il-5*. This corresponds precisely to the localization of *D11Mit1* 30 cM proximal to *Il-5* in an intersubspecific F_2 cross (Dietrich et al. 1992). There were no recombinants between *D11Mit5* and *Myhs,* placing *D11Mit5* 26 ± 1.5 cM distal to *Il-5*. This is more proximal than expected based on the previous localization 13 cM distal to *Il-5* (Dietrich et al. 1992).

Localization of the two new genes, *Irf-1* and *Gabrg-2*, augments the observed synteny homology between human Chr 5q and mouse Chr 11 (Fig. 3). *Pad-1* has been localized on Chr 11 6.1 ± 2.3 cM distal to *Adra-1* and 2.6 ± 1.5 cM proximal to *Il-3* (Buckwalter et al. 1991). This locus has not been mapped in humans. Thus, it is unclear whether the two clusters of human Chr 5q genes (*Adra-1, Gabra-1,* and *Gabrg-2,* and *Il-3, Il-4, Il-5, Csfgm, Irf-1,* and *Sparc*) are part of a continuous conserved segment or are interrupted by a nonhomologous region. This issue is likely to be resolved as additional genes are mapped to Chr 11.

Final distances in cM (± standard deviation) between the loci reported here and all those previously typed on this cross were: *D11Mit1*–9.0 ± 2.7–*Erbb*–12.6 ± 3.2–(*Adra-1, Gabra-1, Gabrg-2*)–4.3 ± 1.9–*Pad-1*–0.9 ± 0.9–*df*–2.6 ± 1.5–(*Il-3/Csfgm, Il-4, Il-5, Sparc, Irf-1*)–2.6 ± 1.5–(*Myhs/D11Mit5*)–1.7 ± 1.2–(*Asgr-1, Rpo-2*)–0.9 ± 0.9–*Edp-1*–5.1 ± 2.2–*Tcf-2*–16.3 ± 4.0–*Erba*–4.5 ± 2.0–*Gh*.

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