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# Localization of the dopamine transporter gene, *Dat1*, on mouse Chromosome 13

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The human dopamine transporter, DAT, plays a central role in the regulation of dopaminergic transmission. DAT removes released dopamine from the synaptic cleft and transports it to presynaptic terminals, thereby terminating dopaminergic neurotransmission. DAT belongs to a family of sodium-dependent neurotransmitter transporters whose members contain putative 12 transmembrane domains. Human disorders such as substance abuse, Tourette's syndrome, and Parkinson's disease may have genetic components and have been implicated in dysfunction of dopaminergic neurons (Pauls and Leckman 1986; Smith et al. 1992). Many abused substances also act on dopaminergic systems. Cocaine binds directly to DAT and inhibits the transport of dopamine into the dopaminergic neurons. This dopaminergic transmission inhibition is thought to be the main mechanism associated with the reinforcing effects of cocaine abuse (Ritz et al. 1987). Localizing the DAT gene in the mouse may be of use in further understanding dopamine-related disorders and the role of DAT in dopaminergic neuron function.

The human dopamine transporter gene, DAT1, has been localized to human Chr 5p15.3 by in situ hybridization (Giros et al. 1992; Vandenbergh et al. 1992). The synteny homology between mouse and human chromosomes suggested that mouse *Dat1* would map to Chr 11, 13, 15, or 18. We extended the linkage analysis of an *M. m. castaneus* intersubspecific backcross (DF/B–*df/df* × CASA/Rk)$F_1$ × (DF/B–*df/df;* Buckwalter et al. 1991) that had been previously characterized for Chr 5, 11, and 18, to include the characterization of Chr 13. We report the localization of *Dat1* in relation to four polymorphic simple sequence repeat markers: *D13Mit10, Il-9* (interleukin-9), *D13Mit8,* and *D13Mit32* (Dietrich et al. 1992).

The simple sequence repeat markers were mapped by PCR amplification with a 96-well MJ machine as previously stated (Dietrich et al. 1992), except that $\gamma^{32}$P-ATP-labeled primers were diluted to a final concentration of 0.7 μCi per reaction, and 100 μg/ml BSA was added to the reaction mixture. Samples were denatured at 92°C for 10 min, cooled to 0°C for at least 5 min, separated by electrophoresis on 6% acrylamide, 8 M urea denaturing sequencing gels, and visualized by autoradiography.

Amplification of the simple sequence repeat markers produced DF/B–*df/df*-specific and CASA/Rk-specific products, which allowed us to create a map of mouse Chromosome (Chr) 13 and link *Dat1* to the chromosome. A *Dat1* RFLP was detected by standard Southern blot analysis. The *Dat1* probe consisted of a 2.3-kb *Eco*RI fragment, from the 5′ region of the rat DAT1 cDNA. Hybridization with this probe revealed a polymorphism in genomic DNA digested with *Bst*EII. The resulting fragments specific for the CASA/Rk allele were 16 and 7.5 kb; those specific for the DF/B–*df/df* allele were 13, 4.5, and 4.2 kb. Fragments of 12 and 6.8 kb were present in both CASA/Rk and DF/B–*df/df* genomic DNA.

We established the unambiguous gene order on the basis of haplotype analysis of 51 backcross progeny (Fig. 1). Using these and additional animals, we calculated the intergenic distance (cM ± estimated standard error) to be: *D13Mit10*–10.7 ± 4.1–*Il-9*–7.2 ± 3.1–(*Dat1, D13Mit8*)–24.4 ± 4.5–*D13Mit32* (Fig. 2). No recombination was detected between *Dat1* and *D13Mit8* in 70 backcross progeny (0 ± 4.2 cM, 95% confidence level). The genetic order and distances we found correlate with previously published results for simple sequence repeat markers (Dietrich et al. 1992).

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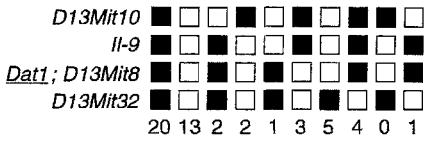


**Fig. 1.** Haplotype distribution of 51 backcross progeny. Each column represents a chromosomal haplotype. The number of animals observed with each haplotype is given below the column. Animals were scored at each locus **(left)** as heterozygous for the *M. m. castaneus* and DF/B-*df/df* alleles (□) or homozygous for the DF/B-*df/df* alleles (■).

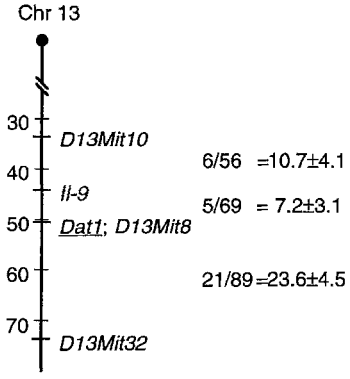


**Fig. 2.** Genetic map of Chr 13. A linkage map of mouse Chr 13 was constructed from the haplotype distribution in Fig. 1. The map distances, in cM, were calculated from the recombinant fraction observed for each interval **(right).** The anchor locus, *Il-9,* was used to assess the position of these loci relative to the centromere **(left,** in cM).

*Dat1* maps to the region of mouse Chr 13 that exhibits an extensive domain of synteny homology with human Chr 5. The nine loci that are the basis for the synteny homology span the entire end of Chr 13, from approximately 40 map units to the telomere. Using the reference locus *Il-9* to compare our map of Chr 13 with the consensus map (Justice and Stephenson 1992), we estimate that *Dat1* maps very close to *Srd5a-1.* This is consistent with human mapping studies that place SRD5A1 on 5p15 and DAT1 on 5p15.3.

The only neuron-specific mouse mutation located in the same region of Chr 13 as *Dat1* is purkinje cell degeneration, *pcd.* Although multiple neuronal cell types die in mice with *pcd* (O'Gorman and Sidman 1985), none are clearly related to dopamine function. In addition, the defect is thought to be intrinsic to the degenerating cells (Mullen 1977) and therefore difficult to directly relate to dopaminergic innervation. Further studies will be necessary to conclusively evaluate *Dat1* as a candidate gene for *pcd.*

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