BRIEF REPORTS

The Human D5 Dopamine Receptor (DRD5) Maps on Chromosome 4

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The human D5 dopamine receptor (DRD5) has been recently isolated and sequenced (Sunahara *et al.*, 1991). It has been shown that this receptor has sequence homology with the D1 dopamine receptor and similarly to the D1 receptor, the D5 receptor stimulates adenylate cyclase activity (Sunahara *et al.*, 1990, 1991). *In situ* hybridization studies showed that the D5 dopamine receptor is neuron specific and that it localizes within limbic regions of the brain. To determine the chromosomal localization of the D5 dopamine receptor, we developed a set of oligonucleotide primers designed to amplify a short DNA fragment from the 3′ untranslated region (3′ UTR) of the gene. Using the polymerase chain reaction we studied the segregation of the amplified products in two human–rodent somatic cell hybrid chromosomal panels. We chose to amplify the 3′ UTR in contrast to the coding sequence because this region tends to be less conserved.

The human–rodent somatic cell hybrid lines used in this mapping protocol were the Bios PCR_{ABLE} DNA from the Bios Corp. (New Haven, CT) and human–rodent somatic cell hybrid mapping panel No. 1 from NIGMS (Camden, NJ). The Bios panel is a human–hamster somatic cell hybrid panel. The NIGMS panel is based primarily on a mouse background, with the exception of one of the lines in this panel, containing human chromosome 9 as its only human chromosome component, which is a human–hamster somatic cell hybrid (Taggart *et al.*, 1985; Mohandas *et al.*, 1986; Warburton *et al.*, 1990; Callen, 1986).

The PCR was performed as described (Saiki *et al.*, 1985), with the following modifications: 60 ng of genomic DNA was used as template for PCR with 80 ng of each oligonucleotide primer, 0.6 unit of *Taq* polymerase, 50 m*M* KCl, 10 m*M* Tris (pH 8.3), 1.5 m*M* $MgCl_2$, 0.01% gelatin, 200 μM each of dGTP, dATP, and dTTP, 2.5 μM dCTP, and 10 μCi of [α-^{32}P]dCTP, in a final reaction volume of 15 μl. The samples were overlaid with 15 μl of mineral oil to prevent evaporation, and the PCR was performed in a Techne MW-1 microplate thermocycler under the following conditions: denaturation at 94°C for 1.4 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min. The cycle was repeated 30 times with a final extension at 72°C for 10 min. The primers used were D5A 5′ CATTTGATTGGTAGTTCGAAG 3′ and D5B 5′ CATAGGATCGTTGGTCCATC 3′, and the expected product was 93 bp long. Two microliters of the reaction mix was electrophoresed on a 6% PAGE sequencing gel, using M13mp18 sequence ladders as sizing markers. The products were visualized by autoradiography. Exposure time was 16 h.

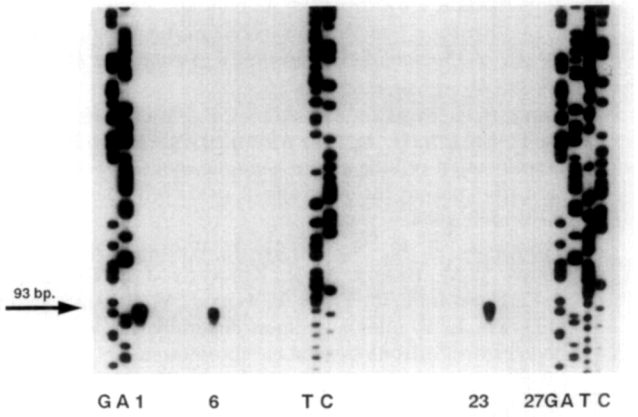

FIG. 1. Autoradiogram for the mapping of the human D5 dopamine receptor. Genomic DNA from the Bios human–hamster somatic cell hybrid mapping panel was amplified as described. The products were visualized by autoradiography. Lanes marked with the letters G, A, T, and C represent M13mp18 sequence size markers. Lane **1** represents the product from total human genomic template, lane **27** represents the product from total hamster genomic template, and the remaining lanes represent the products from different human–hamster somatic cell hybrids. Cell line designations for lanes **2–26** are 324, 423, 734, 750, 803, 860, 940, 212, 507, 683, 756, 811, 983, 862, 909, 937, 854, 904, 967, 968, 1006, 1049, 1079, and 1099. Lanes **1, 6,** and **23,** designated lines UP004, 803, and 1006, respectively, have only chromosome 4 as their common human chromosome.

Analysis of the segregation of the PCR products in the two chromosomal panels was concordant with the presence of human chromosome 4 (Fig. 1). For the Bios hybrid panel, discordancy for the amplified product and chromosome 4 was 0%. For the NIGMS panel the discordancy for the amplified product and chromosome 4 was 17%, and assignment could not be established based only on the results from the NIGMS panel. However, compilation of the segregation of the data from both hybrid panels allowed the assignment of the DRD5 gene to chromosome 4 with an overall discordancy rate of 7%. Discordancy rates for the other chromosomes ranged from 14% for chromosomes 7 and 8 to 51% for chromosome 9. It should be noted that no background from the mouse or hamster templates could be detected in the two hybrid panels used and no additional products were amplified from the human template. The described approach for the mapping of the DRD5 gene could be readily applied to sublocalization efforts.

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A *de Novo* Cystic Fibrosis Mutation: CGA (Arg) to TGA (Stop) at Codon 851 of the CFTR Gene

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The cystic fibrosis transmembrane conductance regulator (CFTR) gene encodes a putative integral membrane protein. Defects in this gene cause the lethal recessive disease cystic fibrosis (CF) (Rosenstein and Langbaum, 1984). The most common mutation in CFTR is a three-nucleotide deletion that removes the phenylalanine residue at position 508 (ΔF508) (Riordan *et al.*, 1989). However, several uncommon mutations that include frameshift, missense, and nonsense mutations have been described. We describe a family in which the single affected child possesses a point mutation that creates a terminator in exon 14A. The patient had severe disease and died at age 12. Neither parent carries this mutation, and analysis with highly polymorphic markers shows that the correct parental samples were obtained. Therefore this is an example of a spontaneous, severe mutation in the CF gene. This class of mutations will further complicate efforts to predict the occurrence of CF by carrier screening or prenatal diagnosis.

We screened 190 non-ΔF508 CF chromosomes for new mutations using the single-stranded conformation polymorphism (SSCP) assay (Orita *et al.*, 1989). We detected a band shift in the PCR product from exon 14A in one individual (1076 from family 1403, data not shown). Direct DNA sequencing of this product indicated that the band shift was the result of a C to T change on one allele at nucleotide position 2683 (CF C2683T). This results in the substitution of an arginine codon (CGA) with a termination codon (TGA) at amino acid position 851, R851X. This mutation is predicted to result in a prematurely truncated protein product. The C to T change at this position destroys a *Taq*I (TCGA) restriction site that was used to confirm the mutation in this individual (data not shown).

Analysis of the parents of individual 1076 revealed a surprising result. A band shift was not detected in exon 14A PCR products from either parent upon SSCP analysis, and *Taq*I digested both alleles in the parents (data not shown), suggesting that the mutation may have arisen *de novo* in the affected individual. However, to make such a conclusion, the parentage of individual 1076 must be firmly established.

The extended family pedigree of individual 1076 is shown in Fig. 1. Affected individuals in family 1407 (the deceased female and 8980) were shown to be carriers for ΔF508, inherited through the father's side of the family. The presence of disease in these siblings indicates that they must also be carriers for another, as yet unidentified, CF muta-

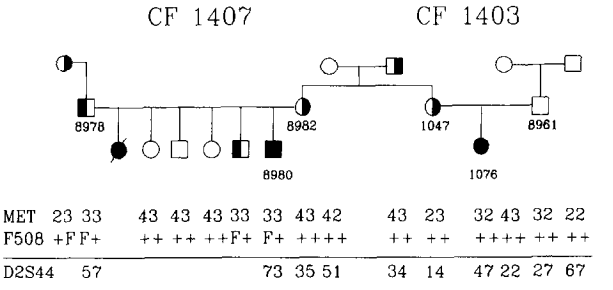

FIG. 1. Pedigree of families 1407 and 1403. Squares designate males and circles designate females. A symbol with a line through it represents a deceased individual. Children are displayed with the oldest on the left. Solid half-filled symbols indicate ΔF508 heterozygotes and the R851X heterozygote in families 1407 and 1403, respectively. Hatched half-filled symbols indicate carriers for the unidentified CF mutation. Haplotype data for *MET* RFLPs (*metD, Msp*I; *metH, Taq*I) are shown below the corresponding individual. Haplotype 2 contains allele 1 of *metD* and allele 2 of *metH;* haplotype 3 is *metD* allele 1, *metH* allele 1; and haplotype 4 is *metD* allele 2 and *metH* allele 2. The data for another polymorphic marker found on chromosome 2 (YNH24, D2S44) (Ref. (3)) are shown below the line. Combined data from polymorphic probes at the D2S44, D3S64, and *MET* locus did not reveal any evidence of nonpaternity. It is estimated that the odds that 8961 is not the father are 10^5 to 1.

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