Polymorphism in the human dopamine D4 receptor gene (DRD4) in Japanese detected by PCR

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Description: There has been reported the polymorphism containing two- to eight-fold repeats of 48-base-pair sequence in the putative third cytoplasmic loop region of dopamine D4 receptor gene (1, 2). Such variation among humans may underlie individual differences in susceptibility to neuropsychiatric disease and in responsiveness to antipsychotic medication (1). In this report, we show the detection of the polymorphism among Japanese population by PCR.

Primer Sequences:
Forward: 5′ ccgcggcctgcagcgctggg 3′.
Reverse: 5′ gcgcggcggctctgacggcg 3′.
PCR products were separated in a 5% polyacrylamide gel.

Frequency: Allele frequency was determined from genomic DNA of 52 unrelated healthy Japanese individuals. The observed heterozygosity was 0.38.

Allele	bp	Number	Frequency
C1	410	1	.01
C2	362	5	.05
C3	314	81	.78
C4	266	1	.01
C5	218	16	.15

Chromosome Assignment: 11p15.5 (3).

Mendelian Inheritance: Co-dominant Mendelian inheritance was observed in 4 unrelated families (18 individuals).

PCR Conditions: One μg genomic DNA, 50 pmole of each deoxynucleotide, 200 mM dNTPs and 5 units Vent (exo$^-$) DNA polymerase (New England Biolabs), 1×Vent buffer (10 mM KCl, 20 mM Tris-HCl (pH 8.8), 0.01% Triton X-100) and 2 mM $MgSO_4$ in a total volume of 100 μl. Fifteen cycles of 99°C for 1 minute and 79°C for 2 minutes followed by 15 cycles of 99°C for 1 minute and 75°C for 2 minutes and finally extension at 75°C for 10 minutes. Both forward and reverse sequences of PCR products of 10 individuals selected randomly were determined by direct sequencing with small modifications (4).

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PCR detection of the TaqA RFLP at the DRD2 locus

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Description, Source and Method: The genomic clone λHD2G1 contains the last coding exon of the human dopamine D2 receptor and approximately 15-kb of 3′ flanking sequence (1). This phage identifies a TaqI RFLP (2). One allele, TaqA1, has been reported to be associated with severe forms of alcoholism (3) and drug abuse (4). As an alternative to Southern blotting we have developed a rapid PCR method for typing individuals with respect to the TaqA RFLP.

λHD2G1 was partially mapped with restriction enzymes and the polymorphic TaqI site was localized to a 5 kb BamHI fragment 3′ of the D2 receptor coding region (5). This fragment was subcloned, digested with TaqI and partial nucleotide sequence from these ends was determined. Based on this sequence a pair of oligonucleotide primers were synthesized and used in a PCR to amplify a 310 bp region (GenBank accesssion no. L22303) surrounding the TaqA site.

PCR Primers:
971: 5′-CCGTCGACGGCTGGCCAAGTTGTCTA-3′
5014: 5′-CCGTCGACCCTTCCTGAGTGTCATCA-3′

Variation: When the 310 bp PCR product is incubated with TaqI the A1 allele remains intact while the A2 allele is cut into a 130 bp piece and a 180 bp piece. All three fragments are resolved on a 1% agarose gel and can be visualized by staining with ethidium bromide. In addition to these three bands a faint band of about 650 bp is also seen in among the PCR products. This band is not cut by TaqI and does not interfere with the analysis.
A1 = 310 bp
A2 = 130, 180 bp

Chromosomal Localization: The phage λHD2G1 was used to map the dopamine D2 receptor gene locus (DRD2) to chromosome 11q22–11q23 (2).

Mendelian Inheritance: The TaqA RFLP is inherited in a Mendelian fashion (2).

PCR Conditions: Performed according to standard conditions; 94°C 1 min, 50°C 1 min, 92°C 1.5 min for 35 cycles. Digestion of 10 μl of the PCR products was accomplished overnight with 5 units of TaqI under oil at 65°C.

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