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Brief Research Communication

Lack of Imprinting of the Human Dopamine D4 Receptor (DRD4) Gene

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The term genomic imprinting has been used to refer to the differential expression of genetic material depending on whether it has come from the male or female parent. In humans, the chromosomal region 11p15.5 has been shown to contain 2 imprinted genes (H19 and IGF2). The gene for the dopamine D4 receptor (DRD4), which is of great interest for research into neuropsychiatric disorders and psychopharmacology, is also located in this area. In the present study, we have examined the imprinting status of the DRD4 gene in brain tissue of an epileptic patient who was heterozygous for a 12 bp repeat polymorphism in exon 1 of the DRD4 gene. We show that both alleles are expressed in equivalent amounts. We therefore conclude that the DRD4 gene is not imprinted in the human brain.

KEY WORDS: genomic imprinting, DRD4, dopamine receptor, polymorphism

INTRODUCTION

Genomic imprinting is a mechanism in mammals whereby alleles of a gene are differentially expressed depending on their parental origin. It has been suggested that imprinting may be involved in expression of disease phenotypes which do not seem to follow normal mendelian inheritance patterns [Hall, 1990]. Findings from a recent study in bipolar I disorder, where phenotypic indicators of the genomic imprinting model were applied to clinical psychopathology data on patients and their families, provide support for the phenotypic expression of genomic imprinting as a mechanism involved in the transmission of this disorder [Grigoroiu-Serbanescu et al., 1995]. A similar study in schizophrenia families failed to provide evidence that genomic imprinting is involved in the expression of the disease. However, due to the sample size only very large effects could be confidently excluded [Asherson et al., 1994].

So far, 7 genes or transcripts have been shown to be imprinted in humans: H19 [Rachmilewitz et al., 1992; Zhang and Tycko, 1992], insulin-like growth factor-II [IGF2; Giannoukakis et al., 1993; Ohlsson et al., 1993], Wilms' tumor suppressor gene [WT1; Jinno et al., 1994], small nuclear ribonucleoprotein polypeptide N [SNRPN; Reed and Leff, 1994], PAR-1 and PAR-5 [Sutcliffe et al., 1994], and IPW [Wevrick et al., 1994]. IGF2 and H19 map to chromosome 11p15.5 [Zemel et al., 1992], a region which is homologous to an established imprinting region on mouse chromosome 7. The observation of a cluster of imprinted genes in this chromosomal region raises the question of whether the imprinted domain is exclusively limited to these genes. It might be possible that parental imprinting functions over a larger chromosomal region and involves other genes on human chromosome 11p15.5 which are located in close proximity to H19 and IGF2. In order to answer this question it is necessary to examine additional genes in this area for monoallelic expression.

In the present study, we investigated the imprinting status of the dopamine D4 receptor (DRD4) gene which is located on chromosome 11p15.5 [Gelernter et al., 1992]. DRD4 is of great interest for research into neuropsychiatric disorders and psychopharmacology since it binds the antipsychotic medication clozapine with higher affinity than does any other dopamine receptor, suggesting that this receptor mediates antipsychotic effects of clozapine [Van Tol et al., 1991]. The gene coding for the DRD4 is highly polymorphic in humans, resulting in various structurally different receptor proteins [Van Tol et al., 1992; Lichter et al., 1993; Catalano et al., 1993; Nöthen et al., 1994; Seeman et al., 1994; Cichon et al., 1995]. To analyze their role in the liability to develop neuropsychiatric disorders and to determine their role in response to medications, studies of linkage and association are being performed. If the DRD4 gene was imprinted this would have essential consequences for the evaluation of these studies; the phenotypic expression of the DRD4 variants would then depend on maternal or paternal inheritance of the variant so that only the expressed allele would have to be taken into consideration.

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In this study, we investigated the expression of DRD4 in brain tissue (tempolateral cortex) of 2 patients (code numbers TB740 and TB762) who had undergone surgery for treatment-refractory epilepsia. We utilized polymorphisms in the coding sequence of the DRD4 gene in order to be able to distinguish between expression of the maternal and paternal allele.

In a first step, genomic DNA extracted from leukocytes [Miller et al., 1988] from probands TB740 and TB762 was tested for heterozygosity for coding variants in exon 1 as previously described [Cichon et al., 1995]. TB762 was identified to be heterozygous for the exon 1 12 bp repeat polymorphism first described by Catalano et al. [1993]. Thus, one DRD4 allele contained 2 repeats of the 12 bp motif and the other allele contained the onefold 12 bp motif. TB740 was homozygous for all tested variants and served as a control.

Subsequently, total RNA was extracted from some 0.5 g of brain tissue from the probands by acid guanidinium thiocyanate-phenol-chloroform extraction [Chomczynski and Sacchi, 1987]. First-strand cDNA was prepared from 1 μg of total RNA using Superscript Preamplification System (Life Technologies) in a volume of 20 μl as specified by the manufacturer. One microliter of cDNA was used as a template for polymerase chain reaction (PCR) amplification of the region containing the 12 bp repeat polymorphism using primers D4ex1.A (5′-ATGGGGAACCGCAGCACC-3′) located in exon 1 and D4ex2.C (5′-CACAGGTTGAAGATGGAGGC-3′) located in exon 2. The PCR reaction was carried out in a 25 μl volume containing 10 pmol of each primer, 200 μM of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 10% glycerol, 5% deionized formamide, and 1 U Taq Polymerase (Life Technologies). After an initial denaturation of 5 minutes at 95°C, 35 cycles of amplification of 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C were performed in a Perkin Elmer 9600 thermocycler. One microliter of PCR product was loaded on a 10% polyacrylamide gel (acrylamide:bisacrylamide = 29:1) containing 1 × TBE. Bands were visualized by silver staining as described [Budowle et al., 1991].

In individual TB762, we observed 2 bands of 380 bp and 368 bp size in equivalent amounts, representing the 2 DRD4 alleles containing the 2-fold 12 bp repeat and the onefold 12 bp repeat, respectively (Fig. 1). Individual TB740 showed only one band of size 380 bp reflecting homozygosity for the 2-fold 12 bp repeat (Fig. 1). Since primers D4ex1.A and D4ex2.C span intron 1, the sizes of the amplification products unambiguously show that they are derived from cDNA and not from genomic DNA.

In a previous study, we observed DRD4 to be expressed from both the maternal and paternal allele in brain tumor tissue [Nöthen et al., 1994]. However, loss or relaxation of imprinting has been proposed as an epigenetic mechanism in a variety of human tumors [Weksberg et al., 1993; Rainier et al., 1993; Ogawa et al., 1993a,b; Suzuki et al., 1994]. Therefore, lack of imprinting in tumor tissues may not guarantee absence of imprinting in normal tissues.

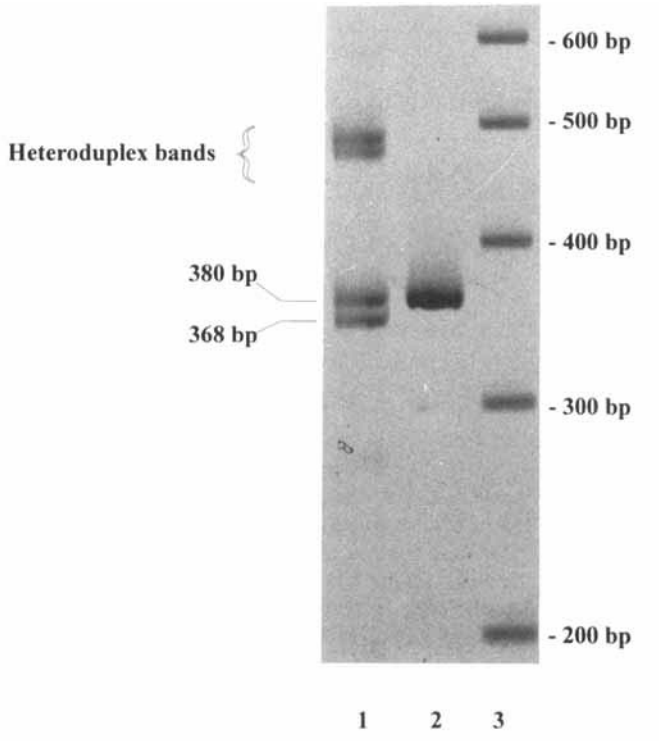

Fig. 1. PCR amplification of the region of the DRD4 cDNA containing the exon 1 12 bp repeat polymorphism using primers D4ex1.A and D4ex2.C. *Lane 1:* Individual TB762 showing 2 fragments 380 bp and 368 bp in length, representing the 2-fold 12 bp repeat and the onefold 12 bp repeat, respectively. *Lane 2:* Individual TB740 showing one fragment of size 380 bp reflecting homozygosity for the 2-fold 12 bp repeat. *Lane 3:* A 100 bp ladder as size marker.

Two other genes located on 11p15.5 have previously been shown to be not imprinted in humans: the gene coding for cathepsin D, which is in close proximity to H19, and the gene coding for placental ribonuclease inhibitor (PRI) which lies more distal, in the vicinity of Harvey ras oncogene (HRAS) and the DRD4 gene [Rachmilewitz et al., 1993].

The present study has 2 major limitations: 1) Imprinting can be a tissue-specific process [DeChiara et al., 1991; Jinno et al., 1994]. Insofar as only neuronal tissue was analyzed, the conclusions need to be restricted to this type of tissue. 2) Imprinting can also be dependent on the developmental stage [DeChiara et al., 1991]. The possibility of imprinting in early phases of development cannot be ruled out from the present study.

In summary, chromosome region 11p15.5 contains a cluster of imprinted genes (H19, IGF2). It is possible that additional genes in this area underlie genomic imprinting. The DRD4 gene, which is an important candidate gene for neuropsychiatric disorders, lies in close proximity to H19 and IGF2. We have approached the question whether the DRD4 gene is imprinted using cDNA from brain tissue of an epileptic person heterozygous for a coding 12 bp repeat polymorphism in exon 1 of the DRD4 gene. We examined for the presence of maternal and paternal allele using the PCR. Both alle-

les were found to be expressed in equivalent amounts. Our results indicate that DRD4 is not imprinted in the human brain.

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