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No Evidence of Association Between Dopamine D_3 Receptor Gene and Bipolar Affective Disorder

M. Paola Piccardi, Giovanni Severino, Alberto Bocchetta, M. Antonietta Palmas, Stefania Ruiu, and Maria Del Zompo*

Department of Neurosciences "B.B. Brodie", University of Cagliari, via Porcell 4, I-09124 Cagliari, Italy

A recent study reported a possible association between allele 1 of the dopamine D_3 receptor gene and bipolar affective disorder using the haplotype relative risk approach. In attempt to replicate these findings, we used similar family-based methods, such as the Haplotype-Based Haplotype Relative Risk method and the Transmission Disequilibrium Test, in a sample of 44 bipolar probands from Sardinia with both parents available. Using the *Bal* I restriction enzyme site polymorphism of Lannfelt et al. (1992), no differences were found between transmitted and non-transmitted alleles and no evidence of linkage disequilibrium was observed. Am. J. Med. Genet. 74: 137–139, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: Haplotype Relative Risk; transmission disequilibrium test

INTRODUCTION

The dopamine D_3 receptor gene (DRD3) is a candidate gene in psychiatric disorders, including affective disorders. The D_3 receptor is characterized by a different tissue distribution compared to dopamine D_1 and D_2 receptors. In particular, D_3 receptor is more widely localized in limbic areas associated with cognitive, emotional and endocrine function implicated in the etiology of affective disorders [Sokoloff et al., 1990; Drevets et al., 1992]. Recent studies focused on an autoreceptor role for D_3 receptor and a distinct pharmacological profile in comparison with other D_2-like receptors [Tang et al., 1994; Griffon et al., 1995]. Lannfelt et al. [1992] identified a point mutation (an A for a G 25 bp downstream from the start codon) in the coding sequence of DRD3 which created a *Bal*I restriction enzyme site. Such a mutation elicits a substitution of *Seryne* by *Glycine* residue at position 9 in the extracellular N-terminal part, which appears to be involved in protein insertion into the membrane. So far, linkage studies concerning *Bal*I RFLP have not supported an involvement of DRD3 in bipolar disorder [Mitchell et al., 1993; Nanko et al., 1994]. Likewise, case-control association studies have afforded negative results [Shaikh et al., 1993; Rietschel et al., 1992]. In a recent study, Parsian et al. [1995] found no evidence of an association between DRD3 alleles and bipolar disorder in a Caucasian sample using a case-control design; however, they reported a specific association between allele 1 of DRD3 and bipolar disorder using the Haplotype Relative Risk (HRR) approach proposed by Falk and Rubinstein [1987]. Moreover, the same study reported a trend towards excess of transmission of allele 1 from heterozygous affected parents. We genotyped a sample of 44 nuclear Sardinian families with bipolar disorder for DRD3 in attempt to replicate Parsian et al.'s findings. Methods such as HRR and other similar ones involving affected-family-based control (parental control genetic association), such as the Haplotype-Based Haplotype Relative Risk (HHRR) method [Terwilliger and Ott, 1992] and the Transmission Disequilibrium Test (TDT) [Spielman et al., 1993] were used.

MATERIALS AND METHODS

Subjects

From a total of 386 outpatients on lithium treatment seen at least once from 1993 to 1995 at the Center of Clinical Psychopharmacology of the Department of Neurosciences, University of Cagliari, 267 met DSM-III-R for bipolar disorder. Of the latter, 44 (32 bipolar I and 12 bipolar II) had both parents available and were thus considered eligible for the present study.

Patients' admission to the study occurred irrespective of family history. All probands and parents were interviewed directly, as were probands' sibs for whom affective disorder was suspected on the basis of family history. Consensus diagnoses according to DSM-III-R [American Psychiatric Association, 1987] were established, independently for probands and relatives by two psychiatrists, derived from the SADS-L structured interview (Schedule for Affective Disorders and Schizophrenia-Lifetime Version) [Spitzer and Endicott, 1978].

*Correspondence to: Prof. Maria Del Zompo, Department of Neurosciences "B.B. Brodie", University of Cagliari, via Porcell 4, I-09124 Cagliari, Italy.

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Of the 44 nuclear families studied, 37 featured one, 6 two, and 1 three members affected by bipolar disorder in generation II. With regard to parents, major affective disorder was diagnosed in 10 fathers (bipolar, 6; unipolar, 4) and 8 mothers (bipolar, 5; unipolar, 3). In one case, both parents suffered from major depression. There were 4 instances of apparent father-to-son transmission of major affective disorder.

Informed consent was obtained from all subjects for participation in the study.

Genotyping

High molecular weight genomic DNA was extracted from whole blood or transformed cell lines [Sambrook et al., 1989]. *Bal*I restriction fragment length polymorphism at the first exon was performed according to the method described by Lannfelt et al. [1992].

The primers used for DNA amplification were 5′-GCT CTA TCT CCA ACT CTC ACA-3′ AND 5′-AAG TCT ACT CAC CTC CAG GTA-3′. PCR reaction contained 150 ng genomic DNA, 2 U Taq polymerase, 1 × Taq polymerase buffer, 0.5 μM each primer, 100 μM dATP, dCTP, dGTP, dTTP, 0.01% gelatin in a total volume of 50 μl. Samples were heated to 95°C for 6 min to denature DNA. Thirty-five cycles were performed according to the following steps: 92°C for 1 min, 56°C for 1 min, 72°C for 1 min, followed by a final extension of 7 min.

The PCR products were subsequently digested with *Msc*I restriction enzyme (isoschizomer of *Bal*I) for 2 hours and the digested products were analyzed on a 3% agarose gel. This polymorphism revealed a 2 allele system, allele 1 was 304 bp in length, allele 2 consisted of two fragments of 206 and 98 bp in length. Two constant bands at 111 and 47 bp were present.

Statistical Analysis

In order to circumvent the problem of a proper control group and to avoid population stratification, we used the HHRR method proposed by Terwilliger and Ott [1992]. This method demonstrated that according to the original HRR design created by Falk and Rubinstein [1987], parental genotypes can be considered independent observations. Thus, the hypothesis that a given allele could be responsible for a putative association with a disease may be tested. Accordingly, the power of analysis may be increased through duplication of available information.

The same data structure is also used in the TDT method [Spielman et al., 1993], but data from more than one affected sib can be used.

Both methods used are insensitive to model of inheritance of illness.

RESULTS

The frequencies of allele 1 and allele 2 in nontransmitted parental chromosomes were 0.64 and 0.36, respectively. No significant departures from the Hardy-Weinberg equilibrium for genotypes in probands (Chi-square = 1.98; 1 df; P = 0.59) were observed. There was no association between DRD3 and bipolar disorder using either the HRR (not shown) or the HHRR method (Table I).

TABLE I. Distribution of Allele Frequencies for DRD3 and Bipolar Disorder (HHRR Method)*

	Allele 1	Allele 2	Total
Transmitted	62	26	88
Not transmitted	56	32	88
Total	118	58	176

*$\chi^2 = 0.92$, df = 1, $P = 0.33$.

Using TDT, 28 families were informative. No linkage disequilibrium was observed between DRD3 and bipolar disorder (Table II).

Among parents with major affective disorder, 16 were heterozygous, and 12 matings were fully informative for study of transmission of DRD3 alleles. In the 12 matings, allele 1 was transmitted 9 out of 12 times. This would result in a significance value of 0.07 testing the null hypothesis of no co-segregation of allele 1 and bipolar disorder using exact binomial test, one-sided.

DISCUSSION

The allele 1 frequency we observed in nontransmitted parental chromosomes (62/88 = 0.64) is within the range (0.59–0.74) found in different Caucasian control samples [Lannfelt et al., 1992; Rietschel et al., 1992 Shaikh et al., 1993; Parsian et al., 1995]. Likewise, the frequency of allele 1 in bipolar probands (0.71) is similar to those reported in previous association studies (0.66–0.73) [Rietschel et al., 1992 Shaikh et al., 1993; Parsian et al., 1995].

Data obtained in the present study using family-based methods, such as HRR, HHRR, or TDT, do not confirm the association between bipolar disorder and allele 1 of DRD3 found by Parsian et al. [1995] when using a similar approach (HRR).

A significant difference was observed between Parsian et al.'s sample and the present one with regard to the proportion of probands with an affected parent (23/28 = 82.1% and 17/44 = 38.6%, respectively). This may be potentially relevant in view of the trends for an excessive transmission of allele 1 observed in both studies in subsamples of families with informative affected parents. Moreover, in an association study of schizophrenia and DRD3, Nimgaonkar et al. [1993] reported that, while there is no overall association, a linkage disequilibrium was observed when the familial cases were analyzed separately.

Since it is unlikely that allele 1, the common form of DRD3, represents the sole or major factor in the development of bipolar disorder, as Parsian et al. [1995]

TABLE II. Transmission Disequilibrium Test (TDT) for Alleles 1 and 2 of DRD3 in Bipolar Disorder

	Number of alleles transmitted		
	Allele 1	Allele 2	Total
Observed	23	18	41

$\chi^2_{tdt} = 0.60$, df = 1, $P = 0.43$.

themselves stressed, we cannot exclude that in our genetically peculiar Sardinian population [Cavalli-Sforza and Piazza, 1993] the presence of different susceptibility factors might mask an association with DRD3.

At this stage, only independent replications of Parsian et al.'s findings in samples from different populations would confirm the relevance of DRD3 in bipolar disorder.

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REFERENCES

American Psychiatric Association (1987): "DSM-III-R: Diagnostic and Statistical Manual of Mental Disorders." 3rd ed., revised. Washington, DC: American Psychiatric Press.

Cavalli-Sforza LL, Piazza A (1993): Human genomic diversity in Europe: a summary of recent research and prospects for the future. Eur J Hum Genet 1:3–18.

Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME (1992): A functional anatomical study of unipolar depression. J Neurosci 12:3628–3641.

Falk CT, Rubinstein P (1987): Haplotype relative risk: an easy reliable way to construct a proper control sample for risk calculation. Ann Hum Genet 12:227–233.

Griffon N, Diaz J, Levesque D, Sautel F, Schwartz J-C, Sokoloff P, Simon P, Costentin J, Garrido F, Mann A, Wermuth C (1995): Localization, regulation, and role of the dopamine D_3 receptor are distinct from those of D_2 receptor. Clin Neuropharmacol 18 (suppl 1):S130–S142.

Lannfelt L, Sokoloff P, Martres M-P, Pilon C, Giros B, Jönsson E, Sedvall G, Schwartz J-C (1992): Amino acid substitution in the dopamine D_3 receptor as a useful polymorphism for investigating psychiatric disorders. Psychiatr Genet 2:249–256.

Mitchell P, Waters B, Vivero C, Le F, Donald J, Tully M, Campedelli K, Lannfelt L, Sokoloff P, Shine J, Selbie L (1993): Exclusion of close linkage of bipolar disorder to the dopamine D_3 receptor gene in nine Australian pedigrees. J Affect Disord 27:213–224.

Nanko S, Fukuda R, Hattori M, Sasaki T, Dai XY, Kanba S, Kato T, Kazamatsuri H, (1994): Linkage studies between affective disorder and dopamine D_2, D_3 and D_4 receptor gene loci in four Japanese pedigrees. Psychiatr Res 52:149–157.

Ningaonkar VL, Zhang XR, Caldwell JG, Ganguli R, Chakravarti A (1993): Association study of schizophrenia with dopamine D3 receptor gene polymorphisms: probable effects of family history of schizophrenia? Am J Med Genet (Neuropsychiatr Genet) 48:214–217.

Parsian A, Chakraverty S, Todd RD (1995): Possible association between the dopamine D_3 receptor gene and bipolar affective disorder. Am J Med Genet (Neuropsychiatr Genet) 60:234–237.

Rietschel M, Nöthen MM, Lannfelt L, Sokoloff P, Schwartz J-C, Lanczik M, Fritze J, Cichon S, Fimmers R, Körner J, Möller H-J, Propping P (1992): A Serine to Glycine substitution at position 9 in the extracellular N-terminal part of the dopamine D_3 receptor protein: no role in the genetic predisposition to bipolar affective disorder Psychiatr Res 46:253–259.

Sambrook J, Fritsch EF, Maniatis (1989): "Molecular Cloning. A Laboratory Manual." Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Shaikh S, Ball D, Craddock N, Castle D, Hunt N, Mant R, Owen M, Collier D, Gill M (1993): The dopamine D_3 receptor gene: no association with bipolar affective disorder. J Med Genet 30:308–309.

Sokoloff P, Giros B, Martres M-P, Bouthenet M-L, Schwartz J-C (1990): Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. Nature 347:146–151.

Spielman RS, McGinnis RE, Ewens WJ (1993): Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52:506–516.

Spitzer RL, Endicott J (1978): Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L). New York: New York State Psychiatric Institute.

Tang L, Todd RD, O'Malley KL (1994): Dopamine D_2 and D_3 receptors inhibit dopamine release. J Pharmacol Exp Ther 270:475–479.

Terwilliger JD, Ott J (1992): A haplotype-based 'haplotype relative risk' approach to detecting allelic association. Hum Hered 42:337–346.