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Brief Research Communication

Association Study Between Two Variants in the DOPA Decarboxylase Gene in Bipolar and Unipolar Affective Disorder

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Irregularities of dopaminergic and serotonergic neurotransmission have been implicated in a variety of neuropsychiatric disorders. DOPA decarboxylase (DDC), also known as aromatic L-amino acid decarboxylase, is an enzyme involved directly in the synthesis of dopamine and serotonin and indirectly in the synthesis of noradrenaline. Therefore, the DDC gene can be considered as a candidate gene for affective disorders. Recently, two novel variants were reported in the DDC gene: a 1-bp deletion in the promoter and a 4-bp deletion in the untranslated exon 1. Subsequently, an association case–control study including 112 English patients and 80 Danish patients with bipolar affective disorder (BPAD) revealed a significant association with the 1-bp deletion. This finding prompted us to analyze whether this effect was also present in a larger and ethnically homogeneous sample of 228 unrelated German patients with BPAD (208 patients with BP I disorder, 20 patients with BP II disorder), 183 unrelated patients with unipolar affective disorder (UPAD), and 234 healthy control subjects. For both BPAD and UPAD we could not detect a genetic association with either variant. Thus, our results do not support an involvement of the 1-bp or 4-bp deletion within the DDC gene in the etiology of affective disorders.
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KEY WORDS: manic-depressive illness; major depression; BPAD; UPAD; DDC

Family, twin, and adoption studies support a major role for genetic factors in the etiology of mood disorders [Reich et al., 1969; Mendlewicz and Rainer, 1977; Tsuang and Faraone, 1990; Craddock and McGuffin, 1993; McGuffin et al., 1994; Nurnberger et al., 1994].

Neurotransmitters such as dopamine, serotonin, and noradrenaline are likely to be contributing significantly to the pathophysiology of affective disorders and have been implicated in the various amine hypotheses of manic depressive illness (bipolar affective disorder; BPAD) and major depression (unipolar affective disorder; UPAD) [van Praag et al., 1970; Samson et al., 1985; Goodwin and Jamison, 1990; Diehl and Gershon, 1992; Owens and Nemeroff, 1994; Maes and Meltzer, 1995; Willner, 1995; Ogilvie et al., 1996]. As known from clinical observations, exposure to dopaminergic drugs might provoke manic symptoms, and exposure

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to antidopaminergic drugs might induce depressive episodes in susceptible individuals [Murphy et al., 1971; Goodwin and Jamison, 1990]. Furthermore, increases in dopaminergic function have been consistently found after long-term treatment with antidepressants [Brown and Gershon, 1993; Bonhomme and Esposito, 1998]. Enzymes involved in the metabolism of these neurotransmitters are of major interest in the search of candidate genes for affective disorders. Recently, Børglum et al. [1999] drew attention to the DOPA decarboxylase (DDC) enzyme, also termed aromatic L-amino acid decarboxylase. DDC (E.C. 4.1.1.28) catalyzes the conversion of L-DOPA to dopamine and 5-hydroxytryptophan to serotonin [Lovenberg et al., 1962; Sumi et al., 1990; Sumi-Ichinose et al., 1992]. DDC is thus involved directly in the synthesis of dopamine and serotonin and indirectly in the synthesis of noradrenaline and adrenaline. Børglum et al. [1999] pointed out that DDC is the sole rate-limiting enzyme responsible for the synthesis of trace amines 2-phenylethylamine, *p*-tyramine, and tryptamine, compounds that act as endogenous modulators of central neurotransmission.

Børglum et al. [1999] described two novel variants in the DDC gene. The first variant is a 1-bp deletion in the DDC promoter and the second one is a 4-bp deletion in the untranslated exon 1. The 1-bp deletion affects a G at position 100 of the sequence presented by Le Van Thai et al. [1993; accession no. L05074], corresponding to nucleotide number g.-601 relative to the transcription start site. The 4-bp deletion comprises a GAGA sequence (position 722–725, sequence accession no. L05074) located in the beginning of a $(GA)_5$ repeat array [Ichinose et al., 1989]. Subsequently, Børglum et al. [1999] tested a potential involvement of these variants in both BPAD and UPAD by means of a genetic association study (case–control design). The study sample comprised 80 Danish and 112 English patients with bipolar disorder, 63 English patients with unipolar disorder, as well as 572 control subjects. Concerning the 4-bp deletion, no significant association in BPAD or UPAD could be observed. In contrast, for the 1-bp deletion, a significant association was found in BPAD, with *P* values varying between 0.021 and 0.037, depending on the tests used. However, Børglum et al. [1999] found no association with this variant in 63 English patients affected by UPAD.

The 1-bp promoter deletion may have a functional impact on the expression of DDC as suggested by Børglum et al. [1999]: The deletion changes a putative binding site for a family of zinc-finger transcription factors, called the NGFI-A family, eliminating their binding capacity. NGFI-A is the prototypic member of a family of immediate-early gene-encoded transcription factors that includes NGFI-C, Egr3, and Krox20, which are known to be expressed in neuronal tissue [Swirnoff and Milbrandt, 1995; Ernø et al., 1996].

The findings by Børglum et al. [1999] prompted us to analyze whether this effect was also present in an independent, larger, and ethnically homogeneous sample of 228 unrelated German patients with BPAD (208 patients with BP I disorder, 20 patients with BP II disorder), 183 unrelated German patients with UPAD, and 234 healthy control subjects. All patients were systematically recruited at the inpatient units of the University Hospital and the Mental State Hospital, both serving the city of Bonn and vicinities. Mean age of interview of patients with BPAD was 42 years (SD = 14) and 50 years (SD = 13) of patients with UPAD. Mean age at onset (defined as the time in life when criteria for a manic, hypomanic, or depressive episode were fulfilled for the first time) was 28 years (SD = 11) for patients with BPAD and 39 years (SD = 13) for patients with UPAD. Lifetime consensus diagnoses according to DSM-IV criteria [APA, 1994] were achieved by trained psychiatrists on the basis of a multidimensional phenotype characterization inventory, including a personal structured interview (Schedule for Affective Disorders and Schizophrenia-L) [Endicott and Spitzer, 1978], family history method (Family Informant Schedule and Criteria) [Mannuzza et al., 1985], Operational Criteria System (OPCRIT) documentation [McGuffin et al., 1991], and a systematic review of medical records. Written informed consent was obtained from all participants before examination. The control group comprised 234 unrelated individuals of German descent (mean age = 30

TABLE I. Allele and Genotype Distribution of Two Deletion Variants in the DOPA Decarboxylase Gene*

	1-bp deletion					4-bp deletion				
	Alleles		Genotypes			Alleles		Genotypes		
Sample	del	wt	del/del	del/wt	wt/wt	del	wt	del/del	del/wt	wt/wt
Patient sample										
BPAD	39	417	1	37	190	138	316	24	90	113
(n = 228/227)	(8.5%)	(91.5%)	(0.4%)	(16.2%)	(83.3%)	(30.4%)	(69.6%)	(10.6%)	(39.6%)	(49.8%)
UPAD	27	339	2	23	158	90	272	14	62	105
(n = 183/181)	(7.4%)	(92.6%)	(1.1%)	(12.6%)	(86.3%)	(24.9%)	(75.1%)	(7.7%)	(34.3%)	(58.0%)
Control sample										
	45	423	0	45	189	128	338	15	98	120
(n = 234/233)	(9.6%)	(90.4%)	(0.0%)	(19.2%)	(80.8%)	(27.5%)	(72.5%)	(6.4%)	(42.1%)	(51.5%)

*The total patient samples were compared with the respective control groups using two global tests on genetic association: Armitage's trend test and a test on allele frequency differences (Fisher's exact test). *P* values were in the range from 0.20 and 0.65. BPAD, bipolar affective disorder; UPAD, unipolar affective disorder.

years, SD = 10). Control subjects were anonymous blood donors for whom ethnicity, year of birth, and sex were known. Patients and controls of non-German descent were a priori excluded in our study.

Polymerase chain reaction (PCR) amplification was performed according to the protocol of Børglum et al. [1999]. After amplification, the PCR products were screened by SSCA analysis [Orita et al., 1989] on a 10% polyacrylamide gel. Bands were visualized by silver staining [Budowle et al., 1991]. PCR products from individuals homozygous for the 1-bp deletion, the 4-bp deletion, and from homozygous (wild-type) control individuals were sequenced and both specific variants described by Børglum et al. [1999] were found. The results are shown in Table I. For both variants, no significant deviation from Hardy-Weinberg equilibrium was detected in both patient groups (BPAD and UPAD) and in the control group. *P* values for Hardy-Weinberg equilibrium deviation are in the range from 0.14 to 0.39 using either the chi-square goodness-of-fit test, or, if there was an expected cell frequency below 5, the exact test according to Elston and Forthofer [1977] was used. Statistical analysis revealed no differential distribution of genotypes or alleles between the patient group affected by BPAD (n = 228) or UPAD (n = 183) and the control group (n = 234) for the 1-bp deletion. Concerning the 4-bp deletion, no differential distribution of genotypes or alleles between the patient groups with BPAD (n = 227) or UPAD (n = 181) and controls (n = 233) could be detected. Following the arguments of Sasieni [1997], Armitage's trend test was used on the 2×3 table of genotype versus phenotype observations. This test is valid and efficient even under deviation from Hardy-Weinberg equilibrium. The test on allele frequency difference between cases and control was calculated as an alternative global test using Fisher's exact test on a 2×2 table.

In conclusion, we were unable to replicate the finding of Børglum et al. [1999] of an association between BPAD and the 1-bp deletion. No association could either be detected for the 1-bp deletion in UPAD or for the 4-bp deletion in BPAD and UPAD. Thus, our results do not support an involvement of the 1-bp or 4-bp deletion within the DDC gene in the etiology of affective disorders. Further studies in large, homogeneous samples are warranted.

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