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Brief report

Sequence analysis of *ADARB1* gene in patients with familial bipolar disorder

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Abstract

Background: The *ADARB1* gene is located in 21q22.3 region, previously linked to familial bipolar disorder, and its product has a documented action in the editing of the pre-mRNA of glutamate receptor B subunit. Dysfunction of glutamatergic neurotransmission could play an important role in the patophysiology of bipolar disorder (BD). Glutamate excitatory neurotransmission regulation is a possible mechanism of the initial effect of anticonvulsants in regulating mood. *Methods*: To investigate the hypothesis of an involvement of *ADARB1* gene in the BD, the *ADARB1* cDNA has been cloned and sequenced in seven selected bipolar I disorder patients with evidence of familiarity of mood disorders. A detailed investigation of the gene nucleotide sequence in the open reading frame has been performed. *Results*: No alteration in the sequence of the *ADARB1* gene cDNA was found in any patient, except a common neutral polymorphism in three out of seven patients. *Conclusions*: Mutations in *ADARB1* gene are not commonly associated with bipolar I disorder, therefore other genes in the 21q22 region could be associated with bipolar illness in some families, likely in the context of a multifactorial transmission model.

Keywords: *ADARB1* gene; Bipolar disorder; Sequencing; Polymorphism; Glutamate; Anticonvulsants

1. Introduction

Population-based epidemiologic studies for mood disorders have stated that bipolar disorders (BD) have a familial relationship (Tsuang and Faraone, 1996; Hook and Palfreyman, 2001). The lack of a Mendelian model of inheritance suggests that multiple loci could be involved in the BD. Genes involved in BD have been searched with the positional cloning approach, as well as with the more direct candidate gene approach. A rational strategy could involve the search for mutations in neurotransmitter-related genes, located in well-established chromosomal locations linked to the BD.

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Table 1
Subject descriptive characteristics ($N=7$)

Patient no.	Sex	Clinical diagnosis	*ADARB1* exon 12 polimorphism (codon 694)	Age	Age at onset	Age at first hospitalisation	No. of hospitalisations	Polarity of the first episode	Rapid cycling	Psychotic episodes	No. of first degree relatives suffering from		
											BPD-I	BPD-II	MDD
17001	F	BPD-I	AAG	35	24	32	6	D	N	N	1	None	1
17011	M	BPD-I	AAG	53	19	19	>10	M	Y	N	None	None	1
17012	M	BPD-I	AAA	27	21	23	3	D	N	N	1	None	1
17014	F	BPD-I	AAG	60	47	47	5	D	N	N	None	None	1
17015	F	BPD-I	AAA	18	13	16	2	D	N	N	1	1	None
17020	F	BPD-I	AAG	58	21	29	>10	D	Y	N	None	1	None
17039	M	BPD-I	AAA	37	22	34	1	D	Y	N	1	None	None

BPD-I, bipolar I disorder; BPD-II, bipolar II disorder; MDD, major depressive disorder.

The *ADARB1* gene appears to be a good candidate for BD (Mittaz et al., 1997) because it is located in a chromosomal region, 21q22, previously linked to familial BD in several independent studies (Straub et al., 1994; Gurling et al., 1995; Detera-Wadleigh et al., 1996, 1997; Smyth et al., 1997; Kwok et al., 1999; Aita et al., 1999; Liu et al., 2001), and its product has a documented influence over the editing, by site-specific deamination, of adenosine of the glutamate receptor B subunit (GluR-B) pre-mRNA (Melcher et al., 1996).

The ADARB1 editing activity on GluR-B pre-mRNA changes, among others, a glutamine (Q) codon in arginine (R) codon, with alteration of the electrophysiologic properties of the GluR-B. It has been hypothesized that alteration in glutamate receptors might alter the risk of developing BD (Schiffer, 2002).

BD has, among the mood disorders, the highest percentage of concordance in monozygotic twins (72%) (Allen, 1976), whose risk of contracting the disease is as much as 75 times greater than that for the general population (Taylor et al., 2002). To investigate the hypothesis of an involvement of *ADARB1* gene in this disorder, we have performed for the first time a sequence analysis of *ADARB1* coding region in seven selected patients with type I BD familiarity.

2. Methods

2.1. Subjects

We selected seven patients aged 18–60 years (Table 1) suffering from a long history of type I BD. The diagnosis was ascertained by the Structured Clinical Interview for DSM-IV. Patients were personally interviewed and evaluated by experienced psy-

Table 2
Primers used for RT-PCR of *ADARB1* cDNAs

No.	Sequence (5′ → 3′)	Region	Product size (bp)
1	GAAACAGTCTCCGCCAGTCAAG	Exon 3 (5′ UTR; F)	575
2	GAAGAGCGTGTCAGGGAAGTCG	Exon 4 (R)	
3	TCGTTCAGTTTCCTAATGCCTCTG	Exon 4 (F)	649
4	GTCATGACGACTCCAGCCAGCAC	Exon 5 (R)	
5	CTCACGCCTGGTCCTGGGTAAGT	Exon 5 (F)	534
6	GTAGTCCCAGCTCCTTGGAAGGTTG	Exon 8 (R)	
7	GTGCAGTGGTGCAATCATGGCTCAC	Exon 8 (F)	452
8	CTGATGCCACTGAGCAAAGGCTTG	Exons 10–11 (R)	
9	GTACCAGCGGATCTCCAACATAG	Exon 10 (F)	488
10	GTTCTGGAGGATGACGCTGGAT	Exon 12 (3′ UTR; R)	

F = forward primer; R = reverse primer; UTR = untraslated region.
See text for details of product size (bp).

chiatrists using Young Mania Rating Scale (Young et al., 1978), Hamilton Depression Rating Scale (Hamilton, 1960), Brief Psychiatric Rating Scale (Ventura et al., 1993), Symptom Check List-90 (Derogatis et al., 1970), and Clinical Global Impression (Spearing et al., 1997).

Diagnostic assessments were based on clinical interviews, review of case notes, and family history data. Starting from 60 bipolar type I italian patients consecutively admitted to the Institute of Psychiatry of Bologna, only seven of them (four females and three males) met stringent clinical criteria for BD type I and for familiarity (Table 1). Relatives were considered affected when they met criteria for BD type I, BD type II or major depressive disorder.

Exclusion criteria were a lifetime diagnosis of schizophrenia, and the presence of somatic or neurological illnesses that would have impaired psychiatric evaluation. The study followed the ethical guidelines laid down by the Declaration of Helsinki (with amendments) and was approved by the local Ethics Committee. All patients gave written consent.

2.2. ADARB1 cDNA sequencing and analysis

Heparinated venous peripheral blood samples (5–10 ml) were drawn from all probands; 1 ml was directly lysed in 5 ml D solution and frozen at −20 °C until total RNA extraction (Chomczynski and Sacchi, 1987).

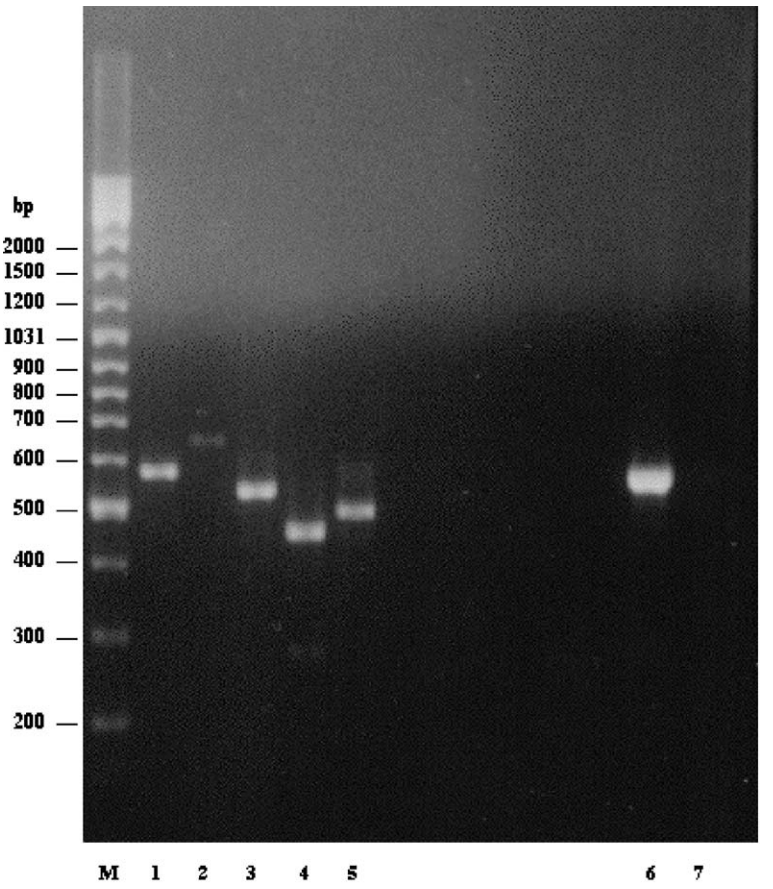

Fig. 1. RT-PCR. Representative examples of *ADARB1* RT-PCR products (1.5% agarose gel stained with ethidium bromide): lane M, GeneRuler marker (Genenco, Florence, Italy); lanes 1–5, products obtained from patient no. 17020 with the five primers pairs (Table 2); lane 6, positive PCR control (primers for housekeeping gene β2M on human placenta cDNA); lane 7, negative control (water).

Total RNA (1 μg) was reverse-transcribed at 42 °C for 60 min in 25 μl final volume by cloned Moloney murine leukemia virus reverse-transcriptase 200 U (Promega, Madison, WI; used with companion buffer), 5 μM oligo dT-15 and 500 μM each dNTP. Five amplification primers pairs (Table 2) were designed to amplify the open reading frame of the longest *ADARB1* mRNA isoform described (DRADA2b, GenBank sequence no. U76421), which also includes the coding sequences of the other known forms generated by alternative splicing (Lai et al., 1997). PCR was performed in 50 μl final volume, containing 5–8 μl RT mix, 1.25 U Taq Polymerase (TaKaRa, Shiga, Japan) with companion reagents (0.2 mM each dNTP, 1.5 mM $MgCl_2$, 1 × PCR buffer), and 0.3–0.4 μM each primer. An initial denaturation step of 2 min at 94 °C was followed by amplification for 40 cycles, (30 s at 94 °C, 30 s at 61 °C, 30 s at 72 °C) and final extension for 7 min at 72 °C.

RT-PCR products were subjected to standard automated sequencing of both DNA strands, with the same primers used in the respective PCR reaction, by BigDye sequencing kit and ABI 310 sequencer (Applied Biosystems, Foster City, CA). The data files were analyzed by Navigator software as well by visual inspection to detect subtle (heterozygotic) polymorphism in both DNA strands.

The sequences of the two detected variants of *ADARB1* mRNA were compared with all known human sequences for *ADARB1* gene in both nonredun-

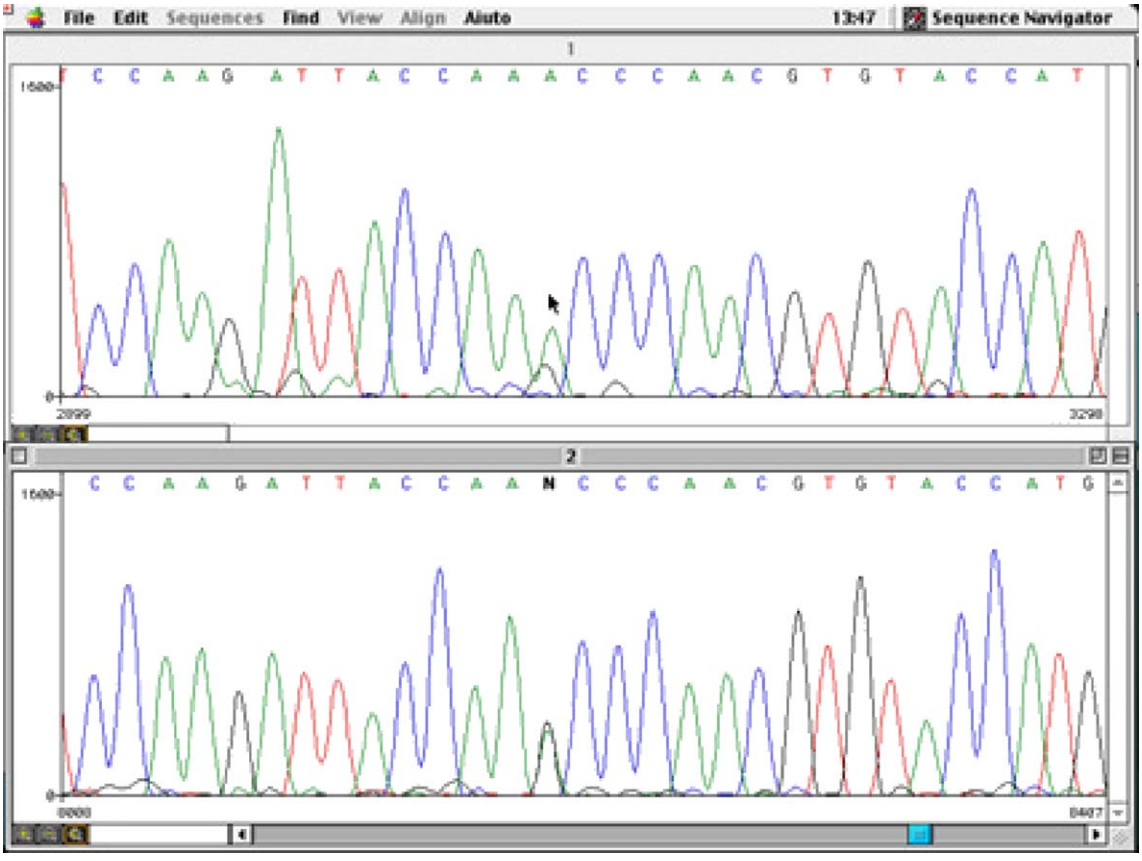

Fig. 2. Sequence analysis. Representative electrophoretogram (Navigator software) of the *ADARB1* cDNA sequence (positions 2482–2509, GenBank no. U76421), patient no. 17012. Upper and lower sets of peaks: sense and antisense strand, respectively. The position 2495 shows heterozygosis (G/A).

dant (nr, finished sequences) and expressed sequence tag (EST) databases, using BLAST2 software (without filter).

3. Results

3.1. ADARB1 cDNA sequencing and analysis

RT-PCR products were successfully obtained from the seven patients. In all cases, gel electrophoresis analysis revealed single bands of the expected size (Fig. 1). This result implies that no gross alteration in the structure and expression capability of the *ADARB1* gene was present in any of these patients.

Analysis of the electrophoretograms showed the presence of an heterozygous nucleotide change of the mRNA coding sequences in three of the seven patients studied (see below), with respect to reference sequence GenBank no. U76421 (Fig. 2).

We detected a G → A transition at position 2495 (in GenBank no. U76421, DRADA2b exon 12) in three patients (nos. 17012, 17015 and 17039 in Table 1). The change was always detected in heterozygosis, along with the described normal reference allele. This base substitution transforms the codon 694 AAG in the codon AAA; in both cases, the coded amino acid is lysine (K). The ''A'' allele was observed in the GenBank sequence nos. U82120, U82121, X99227 and X99383, and it is indicated in dbSNP (NCBI) as cluster rs 1051367.

4. Discussion

The particular editing function of the GluR-B pre-mRNA makes the investigation of *ADARB1* in the BD interesting, because the glutamate is a major mediator in the central nervous system and its regulation has been studied as a possible mechanism of the initial effect of anticonvulsants in regulating mood (Xiaohua et al., 2002). No gross modification of *ADARB1* gene was here found in the seven patients studied, and the mRNA was expressed and correctly spliced in the cells obtained from these patients. In three of the seven patients a nucleotide change was detected in heterozygosis. This change does not likely affect the *ADARB1* function, because, first, it has no effect on the amino acid sequence; moreover, it does not create a cryptic splice site, and abnormal *ADARB1* splicing forms specific for patients bearing the change itself were not detected by RT-PCR; finally, it is also commonly reported in database sequences obtained from normal subjects.

Our data do not formally exclude that in other bipolar patients *ADARB1* mutations or polymorphisms in the noncoding regions could be present, or that *ADARB1* quantitative expression variation in the brain cells could be associated with altered function of GluR-B. Higuchi et al. (2000) have observed that mice homozygous for a targeted functional null allele (*ADARB1* −/−) showed early onset epilepsy and premature death; the impaired phenotype appeared to result entirely from a single under-edited position, the Q/R site, in GluR-B pre-messenger RNA. In humans, alterations of the editing activity of glutamate receptor subunits pre-mRNAs have been found only in a few patients with refractory epilepsy (Grigorenko et al., 1998) and in tissues from malignant human gliomas (Maas et al., 2001), in the absence of a demonstrated sequence variation of *ADARB1* gene.

The negativity of our mutation screening for *ADARB1* in patients with bipolar I disorder adds to previously reported negative results for mutations in the coding region of other candidate genes located on 21q22: synaptojanin-1 gene (SYNJ1), encoding a major presynaptic protein (Saito et al., 2001), and ABCG1, encoding a transporter protein that may be involved in the cellular uptake of tryptophan (Kirov et al., 2001). These data are consistent with a report which suggests that a single major locus in 21q22 does not exist for BD (Vallada et al., 1996).

Other genes on 21q22 were recently candidates for BD, but were still not sequenced: PFKL (Liu et al., 2001), or one of CSTB, APECED, and TMEM1 genes (Yamakawa et al., 1995), on the basis of linkage analysis; PDE9A (Guipponi et al., 1998) and TRPC7 (Nagamine et al., 1998), on the basis of their function related to the nervous system.

Although the patients here studied for *ADARB1* mutation are not high in number, they represent a highly selected sample of subjects with verified type I BD and familiarity for mood disorders. We conclude that mutations in *ADARB1* gene are not commonly associated with type I BD, and that other genes located on 21q22 could be associated with this disease

in some families, likely in the context of a multifactorial model.

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