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BRIEF REPORT

Dopamine receptor D3 gene and response to lithium prophylaxis in mood disorders

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Abstract

Lithium has established itself as an effective prophylactic agent in mood disorders, but not all patients respond to lithium therapy. It is probable that genetic factors play a substantial role in determining the differences in response to lithium. The aim of this study was to investigate the association between the dopamine receptor D3 (DRD3) gene and prophylactic efficacy of lithium in mood disorders. Fifty-five subjects affected by bipolar ($n = 43$) and major depressive ($n = 12$) disorder were followed prospectively for an average of 49 months and were also typed for their DRD3 variant, using polymerase chain reaction techniques. DRD3 variants were not associated with lithium outcome. Consideration of possible stratification effects, such as gender, polarity, family history, age at onset or duration of lithium treatment, also did not reveal any associations. DRD3 variants are not, therefore, a major factor influencing the prophylactic efficacy of lithium in mood disorders.

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Introduction

Lithium has established itself as an effective prophylactic agent in mood disorders but, as not all patients with mood disorders respond to lithium therapy, it is necessary to identify responders prior to treatment. Clinical predictors account for less than 50% of the variance (Goodwin and Jamison, 1990; Maj et al., 1989) and it is probable that genetic factors play a substantial role. A positive family history of bipolar illness has repeatedly been associated with better outcome (Grof et al., 1994; Mendlewicz et al., 1972, unconfirmed by Maj et al., 1998), and lithium-responder probands proved to have a higher genetic loading when compared to non-responders (Mendlewicz et al., 1978). In light of this evidence, lithium response has been used as a tool to select homogeneous samples for association studies with genetic markers (Turecki et al., 1996). Possible genetic predictors of lithium response have not, however, yet been evaluated. Lithium acts by modulating dopamine turnover, although this is via an indirect pathway (Carli et al., 1997). It has been suggested that its efficacy in controlling behaviours related to dopamine stimulants and manic states is mediated by dopamine receptors (Dziedzicka-Wasylewska et al., 1996).

The dopamine D3 receptor (DRD3) gene is a potential candidate as a prediction of lithium response in mood disorders. It is both an autoreceptor and a postsynaptic receptor, and contains a polymorphic site in the first exon that gives rise to a glycine to serine substitution in the N-terminal extracellular domain (Lannfelt et al., 1992). DRD3 expression is mostly localized to a few mesolimbic brain areas and it has a relatively high affinity for dopamine (Schwartz et al., 1993). Antipsychotic treatment has been correlated with a reduction of D3 receptor density in the ventral striatum and its output structures. It also led to a reduction in the number of 5-HT2 receptors in some regions of the prefrontal cortex, suggesting a relationship between the two systems (Joyce et al., 1997). An increase of D2/D3 postsynaptic responsiveness in the mesolimbic system following chronic antidepressant treatment has also been observed (Willner, 1995). The limbic loop interconnecting the prefrontal cortex and ventral striatum may therefore be considered to be the site of regulation of mood symptoms (Joyce et al., 1997). Moreover, DRD3 homo- and heterozygotic genotypes were correlated with significantly different CSF 5-hydroxyindoleacetic acid levels, further suggesting that specific DRD3 genotypes participate in the overall regulation of monoamine turnover in the central nervous system (Jonsson et al., 1996). Finally, DRD3 is mostly localized in the limbic brain regions which are involved in those psychomotor

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abnormalities, such as psychomotor retardation, which have been associated with favourable lithium outcome (Dunner et al., 1976).

The *Bal*I restriction enzyme site polymorphism has been used to reveal evidence for an increased frequency of DRD3*1 and DRD3*1 containing genotypes in transmitted alleles from bipolar families (Parsian et al., 1995), although many opposing results have since been reported (De bruyn et al., 1996; Oruc et al., 1996; Souery et al., 1996). Preliminary results also suggest that the D3 receptor may underlie expression of novelty-seeking in BP patients. Carriers of DRD3 allele 1 showed significantly lower Tridimensional Personality Questionnaire novelty-seeking values when compared to patients without this allele (Staner et al., 1998). Finally, few associations with drug response have been reported, for example, a study on schizophrenia where the DRD3*2/2 genotype was more frequent in patients who did not respond to clozapine (Shaikh et al., 1996), although a negative replication has been reported (Malhotra et al., 1988), and recently DRD3 homozygosity has been associated with neuroleptic response (Krebs et al., 1998).

It was hypothesized that the DRD3 gene could be involved in individual susceptibility to lithium prophylactic efficacy in mood disorders.

Method

Sample

Fifty-five subjects consecutively admitted to the Lithium Clinic for Mood Disorders of S. Raffaele Hospital in Milan were included in this study (male/female = 21(38.2%)/34(61.8%); bipolar/major depressives = 43(78.2%)/12(21.8%)). Our lithium clinic has been involved in treatment of mood disorders and prophylaxis since the early seventies. This has allowed us to collect reliable clinical, psychopathological and time-course information on subjects under standardized treatments, followed prospectively for extended periods (Franchini et al., 1994). Patients considered for this study were part of the sample collected in the context of the European Collaborative Project on Affective Disorders (Souery et al., in press), and a preliminary analysis of the pooled sample has been presented (Lipp et al., 1997). All patients were evaluated using the Schedule for Affective Disorders and Schizophrenia (SADS, Endicott and Spitzer, 1978) and/or the Operational Criteria for Psychotic Illness checklist (OPCRIT, McGuffin et al., 1991). Lifetime diagnoses were assigned by two independent psychiatrists on the basis of interviews and medical records, according to DSM-IV criteria. The presence of concomitant diagnoses of mental retardation, drug dependence, or other Axis I disorders, together with somatic or neurological illnesses that impaired psychiatric evaluation (for example, hypothyroidism mimicking a depressive state) represented exclusion criteria. This allowed only a small part of our subjects to be included in the study. All enrolled patients received lithium as maintenance therapy with doses adjusted to obtain 12-h plasma levels within the standard therapeutic range. The mean values ranged between 0.4 and 0.7-mequiv./l for plasma levels and between 0.2 and 0.4-mequiv./l for red blood cell levels. Determination of lithium levels in each patient was performed every 3 months and, at the same time, the clinical condition of patients was evaluated by M. G. and A. S., using the Hamilton Rating Scale for Depression (21-HAMD, Hamilton, 1967) and Manic Rating Scale for mania (Young et al., 1978). Patients were evaluated at least monthly during active phases of the illness and every 3 months during euthymia. If patients presented a major depressive (HAMD $>$ 18) or manic (Young rating scale $>$ 20) episode, they were recognized as having a new recurrence and received additional care (hospitalization when needed) and treatment according to the judgement of their clinician. All patients received our standard clinical management intervention, involving an explanation about the illness and lithium therapy to the patient and his/her relatives, and monitoring of the course of the illness. Informed consent was obtained from all probands after the procedure had been fully explained; probands were unrelated and of Italian descent, with antecedents from all parts of the country.

The recurrence rates before and during prophylaxis were evaluated by considering the episodes of illness occurring over the months from onset to the beginning of lithium prophylaxis

$$\text{pre-treatment index} = \frac{\text{no. of episodes}}{\text{month duration of illness before lithium treatment}} \times 100$$

and the episodes occurring from the beginning of prophylaxis to the moment of assessment

$$\text{ongoing lithium treatment index} = \frac{\text{no. of recurrences}}{\text{month duration of lithium treatment}} \times 100.$$

The efficacy of prophylactic treatment was evaluated by calculating the difference between the pre-treatment index (PTI) and the ongoing-lithium treatment index (OTI) (Franchini et al., 1994; Gasperini et al., 1993).

DNA analysis

A polymerase chain reaction (PCR) was carried out with the following primers, flanking exon 1 of the D3 receptor

Table 1. DRD3 variants and lithium prophylactic efficacy

DRD3 genotypes	1/1	1/2	2/2	All groups	ANOVA (d.f. = 52,2) *F*	*p*
Number of subjects	29	22	4	55		
Current age (yr)	46.38 ± 13.98	52.0 ± 13.04	51.75 ± 4.92	49.02 ± 13.30	1.22	ns
Onset of illness (yr)	32.28 ± 11.15	35.09 ± 10.78	34.50 ± 9.71	33.56 ± 10.81	0.04	ns
Duration of illness (months)	169.24 ± 127.82	202.91 ± 145.46	207.00 ± 85.06	185.45 ± 131.92	0.46	ns
Pre-lithium treatment recurrence index	6.59 ± 5.58	5.96 ± 5.03	2.45 ± 1.26	6.04 ± 5.22	1.12	ns
Ongoing lithium treatment recurrence index	3.91 ± 6.50	4.55 ± 4.97	3.94 ± 2.16	4.17 ± 5.64	0.08	ns
Pre-ongoing lithium treatment recurring index difference	2.68 ± 8.41	1.41 ± 6.47	−1.50 ± 3.32	1.87 ± 7.41	0.62	ns
Pre-lithium manic and depressive episodes	3.83 ± 2.27	4.95 ± 3.29	3.75 ± 3.10	4.27 ± 2.78	1.11	ns
Ongoing-lithium manic and depressive episodes	1.34 ± 1.54	2.14 ± 2.51	1.75 ± 0.50	1.69 ± 1.96	1.02	ns
Duration of illness before lithium treatment (months)	121.52 ± 115.58	153.27 ± 139.95	155.25 ± 76.41	136.67 ± 123.00	0.46	ns
Duration of lithium treatment (months)	47.72 ± 30.67	49.64 ± 30.02	51.75 ± 20.69	48.78 ± 29.38	0.05	ns

DRD3 variants were not associated with either raw or weighted measurements of lithium prophylactic efficacy. A trend may be observed toward a reduced lithium efficacy with increasing DRD3*2 allele numbers.

gene: 5′-GCTCTATCTCCAACTCTCACA-3′ and 5′-AAGTCTTACTCACCTCCAGGTA-3′ using a 9600 Perkin-Elmer thermocycler. One hundred nanogrammes of genomic DNA was diluted to 12.5 μl using water and heated to 99 °C for 3 min. A reaction mixture was then added, containing 1.5 U sample Taq polymerase, 1 × Taq polymerase buffer, 0.5 mM of each primer, 160 mM of dATP, dCTP, dTTP, dGTP and 0.01% of gelatin in a total volume of 25 μl. Thirty five cycles were performed with a profile of 95 °C for 20 s, 56 °C for 20 s and 72 °C for 20 s. This profile was followed by a 72 °C chase for 4 min. The PCR products were then digested with the *Msc*I restriction endonuclease (*Bal*I isoschizomer, New England Biolabs) overnight and subsequently the digestion products were analysed in 3.5% agarose gel. This polymorphism reveals a two-allele system with frequencies of 0.68 and 0.32, respectively (Lannfelt et al., 1992).

Statistical analysis

Differences were assessed using Analysis of Variance (ANOVA) and post-hoc Newman–Keuls tests. Analysis of Covariance (ANCOVA) was used to include possible confounders. Alpha levels were considered significant when less than 0.05. The power of this sample to detect differences amongst DRD3 variants was calculated considering an α value of 5% two-tailed. With these parameters in the sample, there was a high power (0.80) to detect a large effect size (0.8), that corresponded to a difference of approximately 6 points between the two major genotypes on the difference between PTI and the OTI (Cohen, 1988).

Results

Of the 55 patients, 15 (27.3%) had no DSM IV mood disorder episodes during follow-up and 34 (61.8%) had a decrease in episode frequency. The whole sample showed a significant reduction in episode frequency after lithium treatment (PTI vs. OTI: 6.04 ± 5.22 vs. 4.17 ± 5.64; t = 1.87, d.f. = 54, p = 0.067).

The table shows the sample divided according to DRD3 variants. No association was found either when considering raw or weighted outcome measures. Consideration of possible stratification effects, such as gender, polarity, family history, age at onset, or duration of lithium treatment, also did not reveal any association (data not shown). Subjects were in Hardy–Weinberg equilibrium (χ^2 = 0.01, d.f. = 1, p = 0.95).

Discussion

Dopamine receptor D3 gene was not associated with lithium outcome in this sample of mood disorder subjects. This was also true when known clinical and demographic risk factors, such as polarity, gender, onset, family history and time of illness before lithium administration were

controlled for. To our knowledge, genetic liability factors for lithium response have not yet been studied. This centre is currently testing a number of possible candidate genes within both the dopaminergic and serotoninergic systems.

The DRD3 gene is a possible candidate for prediction of lithium response in mood disorders because its anatomical distribution lies within a site that is considered to be that of regulation of mood symptoms (Joyce et al., 1997), and because it participates in the overall regulation of monoamine turnover in the central nervous system (Jonsson et al., 1996).

The present results do not support this hypothesis, but do not exclude an influence of the dopamine system on depressive symptomatology, since other dopamine receptors remain to be tested. Moreover, regulation of receptor protein expression may influence the overall activity of the system, an effect that is not detected when studying associations at the level of single genes. The present finding confirms our preliminary finding performed in the context of the BIOMED I project, which included part of the present sample (Lipp et al., 1997). In that analysis only the binary variable response/non-response was used as phenotype definition, however DRD3 was excluded as a liability factor for lithium outcome.

It has been argued that most studies, like the present one, may be biased because the selection criteria limit the extent to which the sample is representative (Maj et al., 1998). In fact, lithium prophylaxis may be less effective in routine clinical practice than in specialized treatment centres, possibly due to less rigorous treatment monitoring, non-compliance with medication, complex affective symptoms, and poorer surveillance in detecting recurrent prodromal illness. But the purpose of this study was to enucleate predictive factors and this is more likely when confounding variables, like Axis I comorbidity or substance abuse, are minimized. This selection is also helpful to reduce the possible heterogeneity of mood disorders (Goodwin and Jamison, 1990).

The power of the sample was enough to detect a standardized difference (effect size) up to $d = 0.8$ (depending on the frequency of the DRD3 risk genotype, considering a power of 0.8 and $\alpha < 0.05$ two-tailed), that corresponds to a difference of 6 points on the lithium efficacy index. Indeed, the low power is the main limitation of this article. An effect size of 0.8 may be also expressed as an explained variance of 14%. Therefore it did not prove possible to detect any effect explaining less than 14% of the phenotypic variance. Finally, the power was calculated for the comparison between DRD3*1/1 and DRD3*1/2 subjects; DRD3*2/2 subjects, who had the poorest lithium outcome, are too small a sample to be meaningfully analysed. A limitation of the present study is that a number of clinical variables were not considered, such as number of days of hospitalization, the sequence type of the episode (depression/mania) (Maj et al., 1989), life events or the time-course of plasma lithium levels. With regard to this last point, lithium levels were maintained within range values in our setting and, though high-range serum levels have been associated with better outcome, there have been doubts raised as to their relevance (Gelenberg et al., 1989). Finally, while a very large number of variables intervene in modifying the time-course of mood disorders and psychiatric disturbances in general, the authors share the common view that excluding studies which did not control for all variables would oblige us to ignore a substantial proportion of present knowledge (Solomon et al., 1997).

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