A weak association between TH and DRD2 genes and bipolar affective disorder in a Spanish sample

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Abstract

Genetic factors play an important role in the aetiology of bipolar affective disorder (BP). So far, results of linkage studies have been largely disappointing. We have searched for a possible association between polymorphic DNA markers of two candidate genes (tyrosine hydroxylase, TH; dopamine D2 receptor gene, DRD2) and BP in a population from central Spain. Our results are consistent with the existence of a weak association between these two genes and BP, in such a way that TH and DRD2 could be considered as minor genes contributing to susceptibility.

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Bipolar affective disorder (BP) is a major psychiatric disorder characterised by phases of mania or hypomania alternating with major depressive episodes, which afflicts an estimated 1% of the world's population.[1] Accumulated evidence from family, twin, and adoption studies have shown that genetic factors play an important role in the aetiology of the illness.[2] Lod scores suggesting linkage have been reported using markers on the short arm of chromosome 11,[3] as well as on the long arm of the X chromosome.[4] However, these initial reports have not been reproduced,[5-11] and the finding of a major gene remains elusive. This failure has been attributed to genetic heterogeneity, diagnostic uncertainties, or a not well established mode of inheritance.[12]

In complex diseases, such as major psychoses, the use of the linkage analysis strategy in the search for susceptibility gene(s) has several drawbacks which have been pointed out elsewhere.[13] A strategy additional to linkage analysis in the study of BP is based on studies of association with candidate genes, in which the frequency of an allele at the marker locus in controls is compared with that of the patients.[14-16] Allelic association may be the result of (1) the existence of a disease locus physically close to the marker locus (linkage disequilibrium, that is, recombination frequency <1%), or (2) the marker allele itself is involved in disease risk.

Tyrosine hydroxylase (TH) and the dopamine D2 receptor gene (DRD2) may be considered as good candidate genes for conferring susceptibility to BP. It has long been suggested that a dysfunction of the catecholaminergic system may be involved in the pathogenesis of affective disorders,[1] and tyrosine hydroxylase is a rate limiting enzyme in the synthesis of catecholamines. In addition, Leboyer *et al*[17] reported an association between TH polymorphisms and BP in a French population. On the other hand, it is known that the dopamine antagonists are effective in the acute management of manic phases in bipolar patients, and dopamine agonists seem to be effective antidepressants in some bipolar patients.[1] The dopamine D2 receptor is the major site of action of the neuroleptic haloperidol. Furthermore, three pedigrees have been reported in which translocations close to the chromosomal location of the DRD2 cosegregate with major psychiatric illnesses.[18-20]

We have searched for a possible association between highly polymorphic DNA markers from these two genes and BP in a population from central Spain.

Material and methods

SUBJECTS

We have tested a total of 64 unrelated bipolar affective disorder patients (27 males and 37 females) ascertained at random from admissions to the Psychiatric Service of the Hospital Ramón y Cajal, Madrid, Spain. All of them were diagnosed using the DSM-III-R criteria[21] on the basis of a structured interview, the Structured Clinical Interview for DSM-III-R (SCID),[22] without knowledge of genotypes (by CL and JSR,[2] both experienced clinicians). All bipolar probands met Research Diagnostic Criteria (RDC)[23] for bipolar I (45 subjects) and bipolar II (19 subjects). The patients with schizoaffective disorder were not included in the study. Thirty-three patients (16 males and 17 females) had a family history of affective disorder. The family history was detected using the Family History - Research Diagnostic Criteria (FH-RDC). Forty-nine unrelated subjects (24 males and 25 females) without affective disorder in their family history (defined by affectedness of at least one first degree relative) were recruited from the resources of the Fundación Jiménez Díaz, Madrid, Spain, as control probands. All patients and controls were ethnically white and living in central Spain. The mean ages of the two groups were: controls 40·2 years (SD 12·4), affected subjects 47·1 years (SD 15·4).

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Table 1 Distribution of TH and DRD2 alleles in patients and control subjects

	TH alleles				
	th6	*th7*	*th8*	*th9*	*th10*
Patients (n=60)	34	13	12	18	43
Controls (n=48)	24	11	18	23	20
	DRD2 alleles				
	C1	*C2*	*C3*	*C4*	
Patients (n=51)	10	7	71	14	
Controls (n=46)	13	13	45	21	

Significant differences were obtained by comparing the distribution of TH and DRD2 alleles between patients and controls as deduced from the 2 x 5 contigency table for TH ($\chi^2=9{\cdot}55$, df=4, $p<0{\cdot}05$), and from the 2 x 4 contingency table for DRD2 ($\chi^2=8{\cdot}93$, df=3, $p<0{\cdot}05$).

DNA AMPLIFICATION AND GENOTYPING

DNA was extracted from 10 ml blood samples according to standard methods.[24] A tetranucleotide repeat polymorphism $(CATT)_n$ in the first intron of the TH locus was studied by PCR amplification using the primers and conditions described in Hearne *et al.*[25] A TG dinucleotide polymorphism in the first intron of the DRD2 locus was detected by PCR amplification. The primers used (509 and 419) were those reported by Hauge *et al.*[26] PCR amplification was performed in a total volume of 25 µl containing 200 ng genomic DNA, 10 pmol of each primer, 200 µmol/l of each dNTP, 1·5 mmol/l $MgCl_2$, 50 mmol/l KCl, 10 mmol/l Tris-HCl pH 8·3, 1 unit of DynaZyme DNA polymerase (Finnzymes Inc, Finland), and 0·01% (w/v) gelatin. Amplification was carried out for 30 cycles with denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. PCR products were resolved by electrophoresis on non-denaturing acrylamide gels (12%T, 3%C for TH; 15%T, 3%C for DRD2) and detected by silver staining (Bio-Rad Silver Stain Kit, BIO-RAD).

STATISTICAL ANALYSIS

The statistical significance of the null hypothesis of random association between the marker locus and BP has been measured by the usual χ^2 for contingency tables. The number of alleles in patients and controls were also analysed by Woolf's method.[27] Agreement of genotype frequencies with Hardy-Weinberg equilibrium was evaluated by a χ^2 goodness of fit test. The TH-DRD2 genotype frequencies in controls and patients were also compared using χ^2 analysis.

Results

Fig 1 shows the alleles found for each polymorphism in our sample. A total of five alleles, designated according to the number of tetranucleotide repeats (from 6 to 10), have been detected for the TH polymorphism. The DRD2 polymorphism was found to consist of four different alleles as previously described in other populations.[26]

There appear to be significant differences in the distribution of alleles between controls and patients at both marker loci (table 1). The th10 allele of the TH gene was significantly more frequent in patients (0·358) than in controls (0·208) ($\chi^2=5{\cdot}81$, df=1, $p=0{\cdot}015$). The ratio of subjects with at least one th10 allele was significantly higher in patients (35/60) than in controls (18/48) ($\chi^2=4{\cdot}63$, df=1, $p<0{\cdot}05$). The relative risk of th10 allele was 2·33 ($\chi^2=4{\cdot}559$, df=1, $p=0{\cdot}032$, by Woolf's method[27]). The frequencies of the C3 allele of DRD2 gene were significantly higher in patients (0·696) than in controls (0·489) ($\chi^2=8{\cdot}62$, df=1, $p=0{\cdot}029$). The ratio of patients with at least one C3 allele (47/51) was significantly higher than that of the controls (34/46) (Yates χ^2 corrected

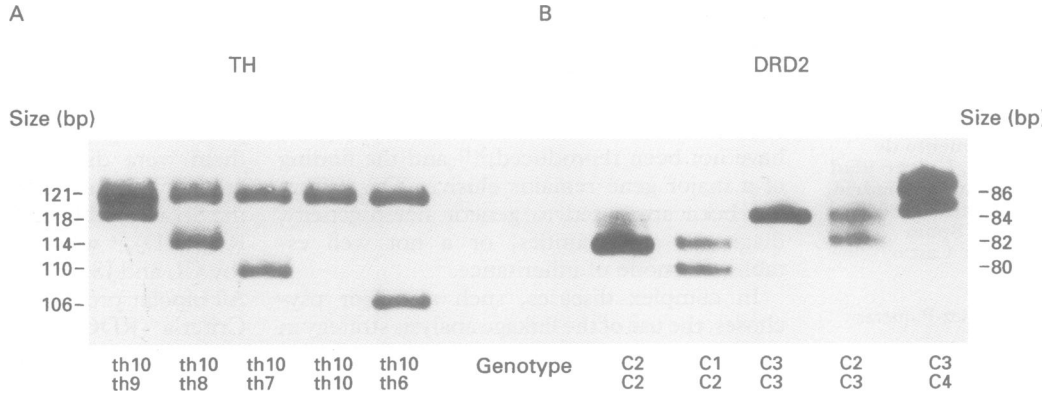

Figure 1 Alleles of the TH (A) and DRD2 (B) polymorphisms that were found in the Spanish sample (Ag staining). Fragment sizes were measured relative to standards.

Table 2 Distribution of DRD2 and TH genotypes in patients and controls

	Genotypes																
TH polymorphism	*th10 th10*	*th10 th9*	*th10 th8*	*th10 th7*	*th10 th6*	*th9 th9*	*th9 th8*	*th9 th7*	*th9 th6*	*th8 th8*	*th8 th7*	*th8 th6*	*th7 th7*	*th7 th6*	*th6 th6*	*HWE* χ^2 (p)*	*P v C† χ^2 (p)*
Patients (n=60)	8	5	6	6	10	2	1	2	6	1	1	2	1	2	7	6·5 (0·7)	14·6 (0·4)
Controls (n=48)	2	3	4	4	5	3	5	1	8	2	0	5	2	2	2	9·7 (0·4)	
DRD2 polymorphism	*C4/C4*		*C4/C3*		*C4/C2*		*C4/C1*		*C3/C3*	*C3/C2*		*C3/C1*	*C2/C2*		*C2/C1*	*C1/C1*	
Patients (n=51)	2		10		0		0		24	4		9	1		1	0	7·3 (0·2)
Controls (n=46)	4		10		1		2		11	6		7	1		4	0	6·2 (0·3) 10·7 (0·2)

* Hardy-Weinberg test.
† Comparison of distribution of genotypes between patients and controls.

Table 3 Distribution of TH-DRD2 genotypes in patients and controls

	TH-DRD2 classes		
Population	*A*	*B*	*C*
Patients (n=46)	22	22	2
Controls (n=45)	11	28	6

TH-DRD2 genotypes were pooled in three classes: (A) subjects with at least one copy of both th10 and C3 alleles, (B) subjects with at least one copy of the th10 or the C3 allele, and (C) subjects without th10 and C3 alleles. The comparison of the patients and controls for these classes showed significant differences ($\chi^2=6{\cdot}38$, df=2, $p<0{\cdot}05$).

for continuity = 4·59, df = 1, $p<0{\cdot}05$). The relative risk of C3 allele was 4·147 ($\chi^2=5{\cdot}268$, df = 1, p = 0·0217, by Woolf's method). The relative risk of the C3 and the th10 alleles was not significant (p = 0·086 and p = 0·15, respectively) after correction by multiplying the p value and the number of polymorphisms.

The genotype frequencies of these markers were in good agreement with those expected under Hardy-Weinberg equilibrium in both polymorphisms for controls and patients. The comparison of genotype distribution for each gene between the two groups did not show significant differences (table 2). The genotype data were analysed in 2 × 2 contingency tables by comparing each genotype with the remaining ones. This analysis failed to reach statistical significance for any genotype at the TH locus. However, the frequency for C3/C3 genotype of the DRD2 polymorphism was significantly higher in patients (0·47) than in controls (0·239) ($\chi^2=5{\cdot}62$, df = 1, p = 0·017). The C3/C3 genotype had a relative risk of 2·828 calculated by Woolf's method ($\chi^2=5{\cdot}454$, df = 1, p = 0·0195), which was not significant after correction ($p>0{\cdot}05$).

A total of 45 controls (22 males and 23 females) and 46 patients (17 males and 29 females) were simultaneously genotyped for TH and DRD2 polymorphisms. All possible TH-DRD2 genotypes were pooled in three classes which were informative for the presence or absence of the alleles th10 and C3 (table 3). The comparison of the tested populations for these classes showed significant differences. The number of subjects with at least one copy of each susceptibility allele (class A) was higher in patients (0·478) than in controls (0·244), while those with at least one copy of C3 or th10 allele (class B), and those without either of the two susceptibility alleles (class C) were more frequent in controls (0·622, 0·113, respectively) than in patients (0·478, 0·043, respectively) (fig 2).

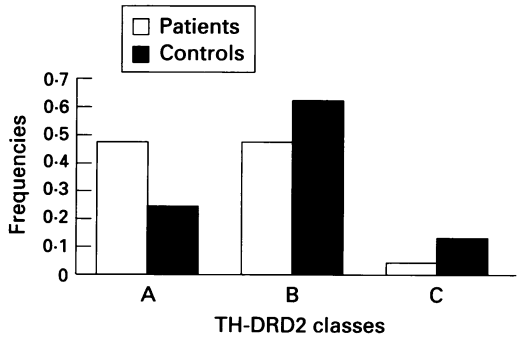

Figure 2 Frequencies of TH-DRD2 genotypes in patients and controls.

Similar results were obtained when the subpopulation of patients with a family history was exclusively considered (data not shown).

Discussion

Initial reports suggesting the involvement of the TH gene in the aetiology of BP have not been reproduced, either by linkage studies (as was mentioned in the introduction to this article) or by association analyses.[12 28–31] Negative results have been also obtained with regard to the DRD2 gene.[11 32–35] However, weak associations of the type we are reporting for TH and DRD2 are very difficult to show by linkage studies, as these analyses assume the existence of a major susceptibility locus, but do not detect the presence of minor modifying genes.[14] In fact, TH and DRD2 have been proposed as modifying genes[9 36] but negative results obtained from genetic association studies could be interpreted if one assumes the existence of genetic heterogeneity.

An advantage of association studies, the strategy used in our work, is that no assumptions need to be made about the mode of inheritance, penetrance, and age of onset of the disease. However, association studies have shown some limitations concerning the size and the stratification of the samples. In this study the stratification has been discarded because both test groups have been matched both for age and ethnic origin.

Regarding the distribution of alleles of the TH and DRD2 polymorphisms, we have found significant differences between control subjects and bipolar affective disorder patients. This result may be interpreted as an association between these genes and BP in our sample. It is worth mentioning that, although a positive association between TH and BP has been previously reported by Leboyer *et al*,[17] this is the first study in which a positive association between DRD2 and BP has been described.

The C3 and th10 alleles were significantly more frequent among patients than controls, even though the relative risk values of these genes suggests a weak association (4·147 for C3 and 2·33 for th10). Hence, these could be considered as minor genes involved in the aetiology of this illness. When the relative risk values were corrected by multiplying by the number of polymorphisms, these values were not significant. However, correction for multiple comparisons is a controversial matter since reducing the type I error for null associations increases the type II error for those that are not null.[37] This undesirable effect could be especially important in our study because the association between TH and DRD2 polymorphisms and BP seems to be moderate in this sample. Furthermore, this correction may be performed to test whether there is an overall association between alleles of a gene and the illness, but not when one wants to test whether there is an association between particular alleles of this gene and the illness, after the demonstration of the overall association com-

paring the distribution of alleles between controls and patients, as performed in our study. On the other hand, the weakness of the association could be because of recombination between these polymorphisms and the mutations responsible for the disease.

Another intriguing finding was that subjects who simultaneously exhibited th10 and C3 alleles were significantly more frequent among patients than among controls. It could be argued that these genes code for two proteins involved in the dopaminergic system. TH catalyses the synthesis of L-Dopa from tyrosine, and L-Dopa is the precursor of catecholamines as dopamine. Thus, alterations in TH could be involved in unbalancing of the normal dopamine production. In addition, modifications of the dopamine receptors, such as DRD2, could be interfering with transmission at dopaminergic synapses in the brain. In the face of these assumptions, the simultaneous implication in most patients of both genes could be in agreement with the existence of an oligogenic model for the illness as has been suggested previously. Moreover, the weakness of the associations indicates that they should be minor genes which behave as additive or modifier genes. However, epistatic interactions cannot be ruled out.

Genetic association with BP was also sought in this sample using the dopamine D4 receptor (DRD4) and α3 subunit of the $GABA_A$ receptor (GABRA3) as candidate genes, but we found no evidence of a positive association[38] (Puertollano *et al*, submitted for publication).

These results provide some evidence for the involvement of dopaminergic systems in the aetiology of manic depression. It remains to be seen whether this hypothesis holds in more extensive studies.

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