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The 5HT1Dβ Receptor Gene in Bipolar Disorder: A Family-based Association Study

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The serotonin (5HT) receptor genes are considered good candidates for Major Depression (MD), Bipolar Disorder (BP), and Obsessive-Compulsive Disorder (OCD). The 5HT1Dβ receptor gene has at least three polymorphisms known: G861C, T-261G, and the functional T371G (Phe-124-Cys). The aim of this study was to investigate for the presence of linkage disequilibrium between the 5HT1Dβ receptor gene and BP. Two hundred and ninety probands with DSM-IV BPI, BPII, or Schizoaffective Disorder (Bipolar type) with their living parents were recruited. Genotyping data for the G861C and T371G polymorphisms were analyzed using the Transmission Disequilibrium Test (TDT). One hundred and sixty triads were informative for the TDT on the G861C polymorphism, which showed no preferential transmission of either allele (chi-square = 0.438, df = 1, p = .508). Only four triads were suitable for the analysis on the T371G variant, with the T allele transmitted once and the G allele transmitted four times to the affected. These findings validate further the results of pharmacological studies excluding a direct involvement of the 5HT1Dβ receptor in the pathogenesis of BP. Further investigations combining genetic and pharmacological strategies are warranted.

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KEY WORDS: *Bipolar disorder; Genetics; Linkage; Transmission disequilibrium test; 5HT1Dβ receptor*

Bipolar Disorder (BP) is a chronic and serious psychiatric condition that affects approximately one percent of the general population and it is characterized by recurrent episodes of depression and mania (American Psychiatric Association 1994). Several studies have pointed out that in the pathogenesis of BP there is a strong genetic component (McGuffin and Katz 1989; Nurnberger and Gershon 1992). BP patients usually experience abnormalities in mood, sleep, sexual behavior, and appetite, functions that are regulated by the serotonin (5HT) system (Meltzer 1989). Moreover, compounds acting on 5HT system (i.e, selective serotonin reuptake inhibitors) have been found to successfully treat bipolar depression (Potter 1998). For these reasons genes of the serotonin system have been considered good candidates for BP (Peroutka 1995) and, thus, have been investigated in several studies. To date, results from these studies are conflicting, but overall seem to exclude the direct involvement of the 5HT receptor genes 5HT2A (Mahieu et al. 1997; Zhang et al. 1997; Vincent et al. 1999), 5HT2C (Gutierrez et al. 1996; Oruc et al. 1997), and of the serotonin transporter protein gene (SLC6A4) (Kelsoe et al. 1996; Oruc et al. 1997; Mendez de Oliveira et al. 1998; Esterling et al. 1998; Vincent et al. 1999; Mundo et al. 2000a) in the pathogenesis of BP. However, a recent meta-analysis study of two 5HTT gene polymorphisms

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in BP and Unipolar Disorder (UP) on more than 1400 individuals of European Caucasian origin showed a mild association of the short allele of the promoter region polymorphism of the SLC6A4 and both BP and UP (Furlong et al. 1998).

The 5HT1Dβ receptor is a terminal auto-receptor involved in the regulation of 5HT release, and it is expressed mostly in the limbic region and in the striatum. It is encoded by an intronless gene located on chromosome 6 (6q14–15) (Demchyshyn et al. 1992). There are at least three polymorphisms known for this gene: the G861C, the T-261G, and the T371G (Lappalainen et al. 1995; Nöthen et al. 1994; Brüss et al. 1999). This last one is quite uncommon, with a prevalence of approximately 2% in the Caucasian population (Nöthen et al. 1994). It causes a substitution of phenylalanine by cysteine in position 124, and recent studies have shown that the Cys-124 variant affects the pharmacological properties of the receptor, reducing the Bmax and increasing the affinity of the receptor for some selective ligands (e.g., sumatriptan) (Brüss et al. 1999; Kiel et al. 2000).

The 5HT1Dβ receptor gene appears to be a good candidate for disorders for which the involvement of 5HT transmission system has been suggested, e.g., Major Depression (MD), BP, and Obsessive-Compulsive Disorder (OCD). To date, only one case-control study on a small sample of subjects has investigated this gene in BP. No significant association was found between the T371G variant and the disease (Brüss et al. 1999). However, a recent family-based association study has shown a linkage disequilibrium between the G861C polymorphism of the gene and OCD, with a preferential transmission of the G allele to the affected (Mundo et al. 2000b).

Case-control genetic association studies may be affected by problems of variable stratification and non-homogeneity of confounding factors, and usually require large samples to give reliable results and to achieve adequate statistical power. On the contrary, family-based association studies are considered better strategies in the identification of genes that may contribute to complex disease susceptibility particularly when the genes are of small effect (Risch and Merikangas 1996), as it is the case for psychiatric disorders.

To test for the presence of linkage disequilibrium, the Transmission Disequilibrium Test (TDT) (Spielman et al. 1993) has been employed. The TDT uses data from families in which marker genotypes are known from the parents and the proband. It calculates the number of transmissions and the number of non-transmissions of the alleles of interest from the heterozygous parents to the affected offspring. The test detects the association between the alleles and the disease in presence of disequilibrium.

The aim of the present study was to investigate for the presence of linkage disequilibrium between the 5HT1Dβ receptor gene and BP. For this purpose we tested two polymorphisms of the 5HT1Dβ gene, the G861C and the T371G (Phe-124-Cys).

METHODS

Sample

Two hundred and ninety probands (113 men, 177 women) affected by Bipolar I (N=196), Bipolar II (N=81), or Schizoaffective Disorder, Bipolar type (N=13), with their living parents were recruited from hospital clinics and newspaper advertisements in Toronto and across Central Canada. Diagnoses were done according to a "best estimate" procedure, and were assessed by a structured interview for DSM-IV (American Psychiatric Association 1994) (SCID-I) administered by trained interviewers blind with respect to the genotypes of the probands.

Two hundred and eighty-four individuals were Caucasian (97.9%), four were Asian (1.4%), and two were Native American (0.7%).

From all patients and their parents, written informed consent to participate in the study was obtained.

Genotyping

Twenty milliliters of blood were drawn into EDTA vacutainers from all probands and their parents. Genomic DNA was extracted using a non-enzymatic procedure (Lahiri and Nurnberger 1991).

G861C

This polymorphism of the 5HT1Dβ gene was genotyped according to the procedure described by Lappalainen et al. (1995). The 548-bp PCR fragment of 5HT1Dβ was amplified using a reaction mixture as follows: 150 ng of genomic DNA, 0.6 μM of each of the primers, forward [5′-gaa aca gac gcc caa cag gac-3′] and reverse [5′-cca gaa acc gcg aaa gaa gat-3′], 1.0 mM magnesium chloride ($MgCl_2$), 0.2 mM dNTP, and one unit of Taq polymerase, with a total reaction volume of 25 μl. An initial denaturation step for 3 min at 95°C was followed by 30 cycles of denaturing at 95°C for 30 sec, annealing at 57°C for 30 sec, and an extension of 72°C for 30 sec. The last cycle was then followed by a final extension step of 72°C for 7 min. All the PCR product was digested with five units of HincII restriction enzyme (New England Biolabs, Mississauga, Ontario, Canada) per reaction at 37°C overnight. The alleles were detected after separation on a 2.5% agarose gel for one hour, and the G allele was the undigested fragment.

T371G (Phe-124-Cys)

This polymorphism was genotyped using the procedure described by Nöthen et al. (1994). The 258-bp PCR fragment of 5HT1Dβ was amplified using a reaction mixture of 150 ng of genomic DNA, 0.8 μM of each of the primers, forward [5′-ccc tac cct gga aag tac tgc-3′] and reverse [5′-tga tgt ccg acg aca gct ag-3′], 1.5 mM magnesium chloride ($MgCl_2$), 0.2 mM dNTP, and one unit of Taq polymerase, with a total reaction volume of 25 μl. Amplification of this fragment was done using the PCR conditions as follow: an initial denaturation step for 5 min at 94°C, followed by 35 cycles of 94°C for 30 sec, annealing at 62°C for 20 sec and elongation at 72°C for 30 sec. After the last cycle, a final extension step at 72°C for 10 min was proceeded. Ten μl of the PCR product was digested with 2.5 units of NheI restriction enzyme (New England Biolabs, Mississauga, Ontario, Canada) per reaction at 37°C overnight. The alleles were separated on a 3.5% high-resolution plus agarose gel (BioShop Canada Inc., Burlington, Ontario, Canada) for approximately 2.5 to 3 hours. The T allele was the undigested band, while the G allele consisted of two bands of 238-bp and 20-bp.

Statistical analyses

To describe the sample clinically in more detail we tabulated mean age, age at onset of BP or Schizoaffective Disorder, and, for BP patients only, presence/absence of seasonal pattern, presence/absence of psychotic symptoms during the mood episodes, and presence/absence of rapid-cycling course.

We tested for the presence of linkage disequilibrium between each of the two polymorphisms of the 5HT1Dβ receptor gene and BP with the Transmission Disequilibrium Test (TDT) (Spielman et al. 1993).

RESULTS

The main demographic and clinical variables of the sample of probands included in the study are summarized in Table 1. No differences were found between genders or among the diagnostic sub-groups for any of the variables tested.

The genotype frequencies for the G861C polymorphism were: GG=51.4%, GC=42.0% and CC=6.6%, while allele frequencies were 72.6% and 27.4% for the G and the C allele, respectively. The sample followed Hardy-Weinberg equilibrium (chi-square = 0.754, p = .37). With respect to this polymorphism, out of the 290 triads comprising the total sample, 160 were informative for the TDT, while the other 130 were triads with homozygous parents. The TDT showed that the G and the C allele of the G861C polymorphism were transmitted equally to the affected subjects (chi-square = 0.438, df = 1, p = .508) (Table 2).

Table 1. Demographic and Clinical Characteristics of the Sample of Patients

	Total (N = 290)	Men (N = 113)	Women (N = 177)
Age	36.3 (10.3)	35.0 (10.8)	37.2 (10.0)
Age at onset	19.7 (7.3)	19.7 (7.0)	19.8 (7.6)
Diagnosis (N):			
BP I	196	81	115
BP II	81	24	57
Schizoaffective Disorder, Bipolar Type	13	8	5
Seasonal Pattern (N)*	11	4	7
Rapid Cycling (N)*	26	7	19
Psychotic Symptoms (N)*	122	53	69

SD are shown in parentheses.
*Only for patients with a diagnosis of BP I or BP II.

In the total sample the genotype frequencies for the T371G (Phe-124-Cys) polymorphism were: TT (Phe-Phe) = 98.5%, TG (Phe-Cys) = 1.5%, and GG (Cys-Cys) = 0%, while allele frequencies were 99.2% and 0.8% for the T and the G allele respectively. For this polymorphism also the sample was in Hardy-Weinberg equilibrium (chi-square = 0.015, p = .90). Only four triads out of the total sample were suitable for the TDT analysis. The T allele resulted to be transmitted only once while the G allele was transmitted four times to the affected (chi-square = 1.8, df = 1, p = .179) (Table 2).

We also performed the TDT considering BP I and BP II triads separately, as some data suggest different vulnerability factors in these two diagnostic sub-groups (Dunner 1998). In our sample there were 107 BP I informative triads for the analysis of the G861C polymorphism, which showed no biases in the transmission of either alleles (chi-square = 1.829, df = 1, p = .176), and four BP I informative triads for the T371G (Phe-124-Cys) polymorphism that were the ones represented in the analysis on the total sample (*see above*). In the 44 BP II informative triads for the G861C polymorphism there were no significant differences in the transmission of the alleles to the affected offspring (chi-square = 0.164, df = 1, p = .685). In the sample of BP II triads there were no informative families for the T371G (Phe-124-Cys) polymorphism.

DISCUSSION

As evidence has implicated the 5HT system in the pathogenesis of BP, our study examined two polymorphisms of the gene encoding for one of the key receptors of 5HT neurotransmission, the 5HT1Dβ.

The main results from this study appear to exclude the direct involvement of the G861C variant of the

Table 2. Results of the TDT on the Two Polymorphisms of the 5HT1Dβ Receptor Genes in the Informative Triads

Variant	Allele	Allele frequency	Transmissions[1]	Non-transmissions[2]	Statistics (chi-square, df, p-value)
G861C	G	.726	88	97	
	C	.274	97	88	0.438, 1, 0.508
T371G	T	.992	1	4	
(Phe-124-Cys)	G	.008	4	1	1.8, 1, 0.179

[1]Number of transmissions from the heterozygous parents to the affected offspring.
[2]Number of non-transmissions from the heterozygous parents to the affected offspring.

5HT1Dβ receptor gene in the pathogenesis of BP, as no biases in allele transmissions were observed. The sample we studied is one of the largest sample of triads of BP probands ever investigated, and its size guarantees reasonable power for the statistical analysis performed (McGinnis 2000). However, with respect to the Phe-124-Cys polymorphism, the Cys-124 allele frequency was very low in our sample. Consequently, the number of triads with heterozygous parents suitable for the TDT analysis was too small to allow us to draw any definitive conclusions on the role of this polymorphism in conferring risk for BP.

Our results are consistent with the negative finding of the other known study investigating the role of the Phe-124-Cys polymorphism in BP (Brüss et al. 1999). In this case-control association study the allele frequencies in the sample of 46 BP probands analyzed were: Phe = 97.8%, and Cys = 2.2%. The frequency of the uncommon allele appeared to be higher than that found in our sample. This was not due to major differences in the ethnicity of the samples because the few non-Caucasian subjects included in our study (2.1%) did not carry the uncommon allele.

With respect to the other polymorphism we investigated, although the G to C substitution of the G861C variant is silent and does not change the aminoacid sequence of the receptor protein, the different alleles could induce different mRNA secondary structure, affecting the efficiency of translation. A recent post-mortem study has suggested differences in the number and in the pharmacological properties of the 5HT1Dβ sites between subjects heterozygous and homozygous for the G variant (Huang et al. 1999). However, further investigations are needed to better clarify this issue.

The fact that the G861C polymorphism of the 5HT1Dβ receptor gene has been found to be associated with OCD in a previous report (Mundo et al. 2000b), while in this study we did not find any association with BP, should be considered with interest.

There is evidence of the critical involvement of 5HT neurotransmission in the pathogenesis of both OCD and BP. This has been derived mostly by data from pharmacological studies showing that SSRIs are successfully used for the treatment of both OCD (Greist et al. 1995; Mundo et al. 1997) and bipolar depression (Potter 1998). However, the 5HT system is complex, and several studies have pointed out that the mechanisms of action of SSRIs in inducing the antidepressant and the antiobsessional clinical responses are substantially different and involve different 5HT receptors (Blier and de Montigny 1998). This suggests that the biological substratum of the two disorders may be different. Other pharmacological studies have supported this distinction leading to the hypothesis that the 5HT1Dβ receptors may be primarily involved in the pathogenesis of OCD, while other 5HT receptors (e.g., the 5HT1A) could be implicated in BP. The acute administration of sumatriptan, a selective agonist of the 5HT1Dβ receptor, induces a transient worsening of obsessive-compulsive symptoms in OCD patients (Zohar 1996), suggesting that a hyper-sensitivity of this receptor may be implicated in the pathogenesis of the disease. The administration of the same compound to patients with unipolar depression results in a blunted response of the growth hormone (GH) (Yatham et al. 1997; Cleare et al. 1998), suggesting a reduced sensitivity of the 5HT1Dβ receptors in these patients. On the contrary, the GH response is not affected by sumatriptan in manic patients or normal controls (Yatham et al. 1997). This last finding points out a biological difference between Unipolar and Bipolar Disorders, and does not support a major role of the 5HT1Dβ receptor in the pathogenesis of BP.

The specificity of the pharmacological findings described above is validated further by the genetic data reported in this study, suggesting that future systematic investigations combining clinical pharmacological and genetic approaches will be quite valuable to elucidating the biological basis of psychiatric disorders.

Finally, although our results are not suggestive of a direct involvement of the 5HT1Dβ receptor gene in conferring risk to BP, it could be hypothesized the implication of the gene in the susceptibility to alternative phenotypes related to BP (e.g., age at onset, suicidal ideation, atypical features). In particular, as the Phe-

124-Cys polymorphism has been found to affect the pharmacological properties of the receptor, it would be interesting to investigate its possible role as a predictor of the pharmacological response to antidepressants.

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