# Analysis and Metaanalysis of Two Polymorphisms Within the Tyrosine Hydroxylase Gene in Bipolar and Unipolar Affective Disorders

Robert A. Furlong,<sup>1,2</sup> Judy S. Rubinsztein,<sup>3</sup> Luk Ho,<sup>3</sup> Cathy Walsh,<sup>3</sup> Tabytha A. Coleman,<sup>1</sup> Walter J. Muir,<sup>4</sup> Eugene S. Paykel,<sup>3</sup> Douglas H.R. Blackwood,<sup>4</sup> and David C. Rubinsztein<sup>1\*</sup>

<sup>1</sup>Department of Medical Genetics, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK <sup>2</sup>Department of Pathology, University of Cambridge, Cambridge, UK

<sup>3</sup>Department of Psychiatry, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK <sup>4</sup>University Department of Psychiatry, Royal Edinburgh Hospital, Edinburgh, Scotland, UK

Tyrosine hydroxylase (TH) is the ratelimiting enzyme in the synthesis of dopamine and noradrenaline. While positive associations between TH and bipolar affective disorder have been found in several studies, many studies have failed to reproduce these results. In order to clarify this situation, association studies of bipolar and unipolar affective disorder groups and metaanalyses of published data on the TH tetranucleotide repeat polymorphism were done. The association studies used the TH tetranucleotide repeat polymorphism in intron 1 and a PstI polymorphism at the 3' end of the gene. The study comprised 124 unrelated bipolar patients, 126 unipolar patients, and 242 controls. There was no significant association of either bipolar or unipolar affective disorder with the TH tetranucleotide repeat polymorphism. However, a weak association ( $\chi^2$ = 3.946, 1 df, *P* = 0.047; odds ratio, allele 2 vs. allele 1 = 0.71 (95% CI, 0.51-0.996)) was observed in the unipolar sample with the TH-PstI polymorphism. Three metaanalyses of published data on the TH tetranucleotide repeat polymorphism in major affective disorder were performed: bipolar I + II vs. control using 583 cases and 745 controls; unipolar vs. control using 204 cases and 359 controls; and bipolar + unipolar vs. control using 846 cases and 823 controls. In each

analysis there was no association of the TH tetranucleotide repeat polymorphism and affective disorder. These results do not support the tyrosine hydroxylase gene having a major role in the etiology of bipolar affective disorder. However, our data suggest that this locus should be examined in larger samples of unipolar affective disorder. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 88: 88–94, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: tyrosine hydroxylase gene; stratified analysis; bipolar affective disorder; unipolar affective disorder

# **INTRODUCTION**

Bipolar affective disorder (or manic-depression) and unipolar affective disorder are common disorders characterized by a disturbance of mood. Twin, family, and adoption studies provide strong support for genetic factors in the pathogenesis of both disorders. As firstdegree relatives of bipolar probands appear to have increased risks for both bipolar and unipolar affective disorders, there may be some factors that are common to both disorders [McGuffin and Katz, 1989; Gershon, 1990].

Tyrosine hydroxylase (TH) catalyzes the ratelimiting step in the synthesis of dopamine and noradrenaline, by converting tyrosine to DOPA (3,4dihydroxyphenylalanine). Together with the serotonergic system, both the dopaminergic and noradrenergic systems have been implicated in the etiology of mood disorders [Willner, 1995]. In addition, dopamine antagonists may be effective in reducing manic symptoms, while dopaminergic drugs such as amphetamine can provoke manic behavior. The human TH gene is localized on chromosome 11p15.5 and spans approximately 8 kb. It consists of 13 primary exons plus two other alternatively spliced exons 5' to "primary" exon 1

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<sup>\*</sup>Correspondence to: David C. Rubinsztein, Department of Medical Genetics, University of Cambridge, Addenbrooke's Hospital, Box 158, Hills Road, Cambridge CB2 2QQ, UK. E-mail: dcr1000@cus.cam.ac.uk

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[O'Malley et al., 1987]. Polymorphisms associated with the TH gene include *RsaI* and *TaqI* restriction fragment length polymorphisms (RFLPs) located in the 5' untranslated region [O'Malley and Rotwein, 1988], *BglII* and *PstI* RFLPs located towards the 3' end of the gene [O'Malley and Rotwein, 1988], and a tetranucleotide repeat within intron 1 [Polymeropoulos et al., 1991].

Several studies using either RFLPs or the tetranucleotide repeat polymorphism have reported a positive association between bipolar affective disorder and tyrosine hydroxylase [Leboyer et al., 1990; Meloni et al., 1995; Perez de Castro et al., 1995; Malafosse et al., 1997], but others have been unable to show any association [Todd and O'Malley, 1989; Korner et al., 1990, 1994; Nothen et al., 1990; Gill et al., 1991; Inayama et al., 1993; Kawada et al., 1995; Souery et al., 1996a,b; Todd et al., 1996; Oruc et al., 1997]. Recently, a metaanalysis of eight studies showed no significant association of the tyrosine hydroxylase gene and bipolar affective disorder. The polymorphisms examined in the study were either the TaqI or PstI RFLPs, or the tetranucleotide repeat polymorphism [Turecki et al., 1997]. However, at least two of the polymorphisms used in this metaanalysis, i.e., the TaqI and PstI RFLPs, are not in complete linkage disequilibrium [Lobos and Todd, 1997]. Since this metaanalysis compared some studies which used only the TaqI polymorphism and one which used only the PstI RFLP, its power would be reduced if only one of these polymorphisms were associated with bipolar affective disorder. Here, we describe association studies using the TH tetranucleotide repeat and the 3' TH-PstI polymorphisms in bipolar and unipolar affective disorders. In addition, metaanalyses of published data on the TH tetranucleotide repeat polymorphism in major affective disorder were performed.

# MATERIALS AND METHODS Polymerase Chain Reaction

Polymerase chain reaction (PCR) of the TH tetranucleotide repeat polymorphism [Polymeropoulos et al., 1991] was performed using the primers TH-TETF (5'CAG CTG CCC TAG TCA GCA C 3') and TH-TETR (5' GCT TCC GAG TGC AGG TCA CA 3'). Approximately 50-200 ng of DNA were amplified in a 10-µl reaction volume containing 10 mM Tris-HCl, pH 8, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM of dGTP, dATP, and dTTP, 40 μM of dCTP, 0.75 μCi of α<sup>32</sup>P-dCTP, 50 ng of each primer, and 0.5 units of Taq polymerase (GIBCO BRL, Paisley, UK). PCR conditions were 94°C for 4.5 min, followed by 30 cycles of 94°C for 0.5 min, 58°C for 0.5 min, 72°C for 0.5 min, and 72°C for 5 min. Products were electrophoresed on 6% sequencing gels and autoradiographed. Alleles were scored as alleles 1 (260 bp) to 6 (240 bp).

Amplification of the TH *Pst*I/PCR polymorphism (Genome DataBase) was performed using the primers TH.*Pst*F (5' GCC TCG GAT GAG GAA ATT GAG AAG C 3') and TH.*Pst*R (5' GCT GTC CAG CAC GTC GAT GGC C 3'); the 5' end of each primer corresponded to positions 1091 and 1411 of the TH cDNA sequence

[Grima et al., 1987], respectively. Each 10-µl reaction contained 1 µl of DNA (50–200 ng), 10 mM Tris-HCl, pH 8, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 50 ng of each primer, and 0.5 units of *Taq* polymerase. PCR conditions were an initial denaturation at 94°C for 4.5 min, followed by 30–35 cycles of 94°C for 0.5 min and 71°C for 1.5 min, and a final extension at 72°C for 5 min. Products were digested with 10 units of *PstI* at 37°C for 2 hr and electrophoresed on 1% agarose gels stained with ethidium bromide. Bands sized approximately 1.45 kb and 0.80/0.65 kb were scored as alleles A and B, respectively.

The genotype frequency of either the TH tetranucleotide repeat polymorphism or the TH *PstI* polymorphism in the control groups (see Tables II and III) did not differ significantly from those expected from the allele frequencies, on the basis of Hardy-Weinberg equilibrium assumptions (TH tetranucleotide repeat polymorphism  $\chi^2 = 2.56$ , 14 df, P = 1.00 (rare genotype 5 6 omitted from analysis); TH-*PstI* polymorphism, Cambridge sample:  $\chi^2 = 0.510$ , 2 df, P = 0.77; Edinburgh sample:  $\chi^2 = 0.324$ , 2 df, P = 0.85).

Power calculations for the TH tetranucleotide repeat polymorphism show that with observed allele frequencies >0.2 in the control group, we should be able to detect a gene with a relative risk of >2.0. For the TH-*Pst*I polymorphism, power calculations show that, for a significance level of 5% and 80% power and an observed allele frequency of approximately 0.5, in the Cambridge control group a gene with a relative risk of >2.0 should be detected [Breslow and Day, 1980].

## **Patients: Cambridge**

One hundred and twenty-four unrelated bipolar patients (56 male, 68 female) and 126 unipolar patients (47 male, 79 female) were used in the study. Control DNA comprising 242 anonymous unrelated individuals (90 male and 132 female) from an East Anglian population was obtained from the Molecular Genetics Laboratory, Addenbrooke's Hospital DNA Bank. Referral patterns to this laboratory suggest that at least 97% of controls were Caucasian, about 80% had parents who were East Anglian, and virtually all of the remainder were from the UK. The surnames of controls were screened in order to exclude individuals who were obviously of non-Caucasian origin. It is important to note that this region of the UK includes a large rural catchment area and is not characterized by the ethnic variation seen in many large cities. Ethical approval for genetic studies of bipolar and unipolar affective disorder was obtained from local ethics committees. The bipolar and unipolar patients were recruited from inpatient and outpatient clinics in East Anglia and were English Caucasian in origin, i.e., both parents were English. The patients were assessed by trained clinicians using the SADS-L interview [Endicott and Spitzer, 1978] supplemented by case note review, meeting Research Diagnostic Criteria for bipolar affective disorder I or major affective disorder [Spitzer et al., 1978]. The inclusion criteria for the unipolar sample comprised an

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TABLE I. Summary of Studies Used in Metaanalyses of Tyrosine Hydroxylase Tetranucleotide Repeat Polymorphism

Study	Authors	Ethnic origin	Cases and controls	Interview and diagnostic criteria; control group
1	Furlong et al., present study	English Caucasian	120 bipolar I 125 unipolar 239 controls	SADS-L using Research Diagnostic Criteria; controls were unscreeded for affective disorders
2	Oruc et al., 1997	Croatian	42 bipolar I 44 unipolar 70 controls	SADS-L diagnosis, using Research Diagnostic Criteria; controls interviewed using SADS-L
3	Souery et al., 1996b	Belgian	66 bipolar 67 controls	SADS-L diagnosis, using Research Diagnostic Criteria; controls had no family or personal history of affective disorders
4	Meloni et al., 1995	French	47 bipolar I 17 bipolar II 64 controls	French version of the Diagnostic Interview for Genetic Studies Controls had no family or personal history of affective disorders
5	Perez de Castro et al., 1995	Spanish white	60 bipolar I + II 48 controls	DSM-III-R Research Diagnostic Criteria for bipolar I and II; controls had no family history of affective disorder.
6	Korner et al., 1994 (Bonn)	German	64 bipolar 104 controls	SADS-L, using DSM-III-R Research Diagnostic Criteria; controls were unscreened for affective disorders
7	Korner et al., 1994 (London)	British	53 bipolar 51 controls	SADS-L, using DSM-III-R Research Diagnostic Criteria; controls were unscreened for affective disorders
8	Korner et al., 1994 (Cardiff)	British	66 bipolar 54 controls	SADS-L, using DSM-III-R Research Diagnostic Criteria; controls were unscreened for affective dioorders
9	Souery et al., 1996a	Belgian	70 bipolar 35 unipolar 50 controls	SADS-LA, using Research Diagnostic Criteria; controls interviewed using SADS-LA
10	Cavazzoni et al., 1996	Canadian Caucasian	48 bipolar 6 unipolar 94 controls	Research Diagnostic Criteria for major affective disorder, bipolar or recurrent unipolar subtype; controls were unscreened for affective disorders
11	Todd et al., 1996	North American white	49 bipolar + both parents	Meeting DSM-III-R diagnostic criteria Haplotype relative risk nontransmitted alleles of the parents were considered as "control" alleles

age of onset of the first episode of depression between 18–65 years, at least two episodes of unipolar major depression, and at least one inpatient admission for the treatment of depression.

## **Patients: Edinburgh**

Eighty-nine unipolar cases (36 male, 53 female) and 206 anonymous control subjects were used in the study. All patients and controls were Caucasian and were domiciled in Scotland. Patients were assessed using the SADS-L interview and case note review, and met Research Diagnostic Criteria for major depressive disorder. All had two or more episodes of illness. All control samples (age range, 18-60) were collected from the Edinburgh Blood Transfusion Service within the same catchment area as the patient sample. They were screened to exclude anyone taking any sort of medication. In addition, donors answered a screening questionnaire that would result in exclusion of major mental illness, though in many cases that would probably not exclude past episodes of unipolar depression or those with a family history of affective disorders.

## **Metaanalyses**

Studies of the TH tetranucleotide repeat and affective disorder were considered for inclusion in metaanalyses following a Bath Information and Data Services (BIDS) literature search on March 26, 1998 (Release 7.1.00, previously updated on March 25, 1998). Twelve studies were identified: the present study, Oruc et al. [1997], Cavazzoni et al. [1996], Souery et al. [1996a,b], Todd et al. [1996], Kawada et al. [1995], Meloni et al. [1995], Perez de Castro et al. [1995], and the Bonn, London, and Cardiff studies published in Korner et al. [1994]. The Japanese study of Kawada et al. [1995] was omitted from the analyses, as it is unclear which of the six alleles detected in that study correspond to the five common alleles found elsewhere. The study of Todd et al. [1996] used the haplotype relative-risk method. Alleles transmitted to affected cases were considered "bipolar" alleles, while the nontransmitted alleles in the

TABLE II. Tyrosine Hydroxylase Tetranucleotide Repeat Polymorphism in Cambridge Control, Bipolar, Unipolar, and Bipolar + Unipolar Affective Disorder Groups\*

Tetranucleotide repeat	Control (%)	Bipolar (%)	Unipolar (%)	Bipolar + unipolar (%)
Allele 1	162 (33.9)	62 (25.8)	101 (40.4)	163 (33.3)
Allele 2	68 (14.2)	36 (15.0)	33(13.2)	69 (14.1)
Allele 3	52 (10.9)	33 (13.8)	25 (10.0)	58 (11.8)
Allele 4	88 (18.4)	52(21.7)	47 (18.8)	99 (20.2)
Allele 5	107(22.4)	57(23.8)	43(17.2)	100 (20.4)
Allele 6	1(0.2)	0 (0.0)	1(0.4)	1(0.2)
Total	478	240	250	490

\*For bipolar vs. control: alleles,  $\chi^2 = 5.50$ , 4 df, P = 0.24; genotypes,  $\chi^2 = 17.08$ , 14 df, P = 0.25; for unipolar vs. control: alleles,  $\chi^2 = 4.33$ , 4 df, P = 0.36; genotypes,  $\chi^2 = 17.69$ , 14 df, P = 0.22; for bipolar + unipolar vs. control: alleles,  $\chi^2 = 1.07$ , 4 df, P = 0.90; genotypes,  $\chi^2 = 12.54$ , 14 df, P = 0.56.

TABLE IIIa. Tyrosine Hydroxylase *Pst*I Polymorphism in Cambridge Control, Bipolar, Unipolar, and Bipolar + Unipolar Affective Disorder Groups, and Edinburgh Control and Unipolar Groups\*

		Cambri	dge sample (%)	Bipolar +	Edin	Edinburgh sample (%)	
TH <i>Pst</i> I	Control (%)	Bipolar	Unipolar	unipolar (%)	Contro	l Unipolar	
Allele A	195 (49.2)	109 (46.6)	119 (57.8)	228 (51.8)	52 (54.2	2) 40 (60.6)	
Allele B	201(50.8)	125(53.4)	87 (42.2)	212(48.2)	44 (45.8	B) 26 (39.4)	
Total	396	234	206	440	96	66	
Genotype AA	53 (26.8)	27(23.1)	34 (33.0)	61(27.7)	15 (31.3	15 (45.4)	
Genotype AB	89 (44.9)	55(47.0)	51 (49.5)	106 (48.2)	22(45.8)	3) 10 (30.3)	
Genotype BB	56 (28.3)	35 (29.9)	18 (17.5)	53(24.1)	11(22.9)	) 8 (24.2)	
Total	198	117	103	220	48	33	
				Cambridg	e		
	Cambri	idge	Cambridge	bipolar + uni	oolar	Edinburgh	
bipolar vs.		control,	unipolar vs. control,	vs. control	l,	unipolar vs. control,	
TH PstI odds ratios (9		(95% CI)	odds ratios (95% CI)	odds ratios (95	% CI)	odds ratios (95% CI)	
Alleles: B vs. A	lleles: B vs. A 1.11 (0.8–1.54)		0.71 (0.51-0.996)	0.90 (0.69–1.19)		0.77 (0.41-1.45)	
Alleles: AB vs. AA 1.22 (0.68–1.49)		0.89 (0.51-1.55)	1.03 (0.65–1.64) 0.45 (		0.45 (0.16-1.28)		
Alleles: BB vs. AA	1.22(0.65)	-2.27)	0.50 (0.25-0.993)	<b>0.50 (0.25–0.993)</b> 0.82 (0.49–1.39)		0.73(0.23 - 2.32)	

\*Statistically significant results are shown in boldface. For Cambridge bipolar vs. control: alleles,  $\chi^2 = 0.417$ , 1 df, P = 0.52; genotypes,  $\chi^2 = 0.530$ , 2 df, P = 0.77; for Cambridge unipolar vs. control: alleles,  $\chi^2 = 3.946$ , 1 df,  $\mathbf{P} = 0.047$ ; genotypes,  $\chi^2 = 4.436$ , 2 df, P = 0.11; for Cambridge bipolar + unipolar vs. control: alleles,  $\chi^2 = 0.553$ , 1 df, P = 0.46; genotypes,  $\chi^2 = 0.971$ , 2 df, P = 0.62; for Edinburgh unipolar vs. control: alleles,  $\chi^2 = 0.661$ , 1 df, P = 0.42; genotypes,  $\chi^2 = 2.274$ , 2 df, P = 0.32.

parents were considered "control." The rare allele 6 (allele F) reported in the present study and by the study of Todd et al. [1996] was omitted from analyses. A summary of the details of each study included in metaanalyses is shown in Table I. Three analyses were performed.

Analysis 1: Bipolar I and II vs. Control. Studies included: the present study, Oruc et al. [1997], Souery et al. [1996b], Todd et al. [1996], Meloni et al. [1995], Perez de Castro et al. [1995], and the Bonn, London, and Cardiff studies in Korner et al. [1994].

Analysis 2: Unipolar vs. Control. Studies included: the present study, Oruc et al. [1997], and Souery et al. [1996a].

Analysis 3: Bipolar + Unipolar vs. Control. Studies included: the present study, Oruc et al. [1997], Cavazzoni et al. [1996], Todd et al. [1996], Souery et al. [1996a], Meloni et al. [1995], Perez de Castro et al. [1995], and the Bonn, London, and Cardiff studies in Korner et al. [1994]. Study 9 [Souery et al., 1996a], which included patients with both bipolar and unipolar disorder, was included in this analysis in preference to study 3 [Souery et al., 1996b], which reported results with bipolar cases alone: it is likely that some of the cases and controls were common to both studies.

Information concerning TH tetranucleotide genotypes in the selected studies was incomplete, and thus was not analyzed.

# **Statistical Analysis**

Metaanalyses and calculation of odds ratios with 95% confidence intervals were determined using unconditional logistic regression, with the general loglinear analysis option of the SPSS version 6.0 software package (SPSS, Inc., Chicago, IL). This form of analysis is robust to differences in control allele frequencies in different studies.

#### RESULTS

# Tyrosine Hydroxylase Tetranucleotide Repeat Polymorphism: Cambridge Data

Six TH tetranucleotide repeat alleles were detected (Table II). The rare allele 6 was omitted from subsequent analyses. No significant differences were found when the control allele or genotype frequencies were compared to those in the bipolar (alleles:  $\chi^2 = 5.499$ , 4 df, P = 0.24; genotypes:  $\chi^2 = 17.079$ , 14 df, P = 0.25), unipolar (alleles:  $\chi^2 = 4.326$ , 4 df, P = 0.36; genotypes:  $\chi^2 = 17.691$ , 14 df, P = 0.22), or combined bipolar + unipolar (alleles:  $\chi^2 = 1.073$ , 4 df, P = 0.90; genotypes:  $\chi^2 = 12.535$ , 14 df, P = 0.56) groups.

# Tyrosine Hydroxylase PstI polymorphism

For the TH-*Pst*I polymorphism, 117 bipolar patients, 103 unipolar patients, and 198 controls were successfully genotyped from the Cambridge sample, while results were obtained with 48 controls and 33 unipolar patients from the Edinburgh sample (Table III). In the Cambridge sample there were no significant differences when the control allele or genotype frequencies

TABLE IIIb. Tyrosine Hydroxylase *Pst*I Polymorphism: Stratified Analysis of Cambridge + Edinburgh Unipolar Samples\*

	Unipolar vs. control			
TH PstI	$\chi^2$	df	Р	
Alleles				
Test of heterogeneity between studies	0.047	1	0.83	
Test of association	4.621	1	< 0.05	
Alleles: B.S. vs. A, odds ratio (95% CI)	0.72 (0.54-0.97)			
Genotypes				
Test of heterogeneity between studies	2.660	<b>2</b>	0.27	
Test of association	4.237	<b>2</b>	>0.20	
Genotypes: AB vs. AA, odds ratio (95% CI) Genotypes: BB vs. AA, odds ratio (95% CI)	0.78 (0.48–1.26) <b>0.55 (0.30–0.98)</b>			

\*Statistically significant results are shown in boldface.

were compared to those in the bipolar (alleles:  $\chi^2$  = 0.417, 1 df, P = 0.52; genotypes:  $\chi^2 = 0.530$ , 2 df, P = 0.570.77) or combined bipolar + unipolar (alleles:  $\chi^2$  = 0.553, 1 df, P = 0.46; genotypes:  $\chi^2 = 0.971$ , 2 df, P =0.62) groups. However, a statistically significant result was obtained when unipolar and control allele frequencies were compared ( $\chi^2 = 3.946, 1 \, df, P = 0.047$ ), giving an odds ratio of 0.71 (95% CI = 0.51-0.996) for allele B vs. allele A. Comparison of unipolar and control genotypes ( $\chi^2 = 4.436$ , 2 df, P = 0.11) gave an odds ratio of 0.50 (95% CI = 0.25-0.993) for genotype BB vs. genotype AA. Although this result is not significant, after correction for multiple testing, we sought to examine this phenomenon further in an independent set of cases and controls from Edinburgh. No appreciable differences were found in the Edinburgh unipolar and control groups (alleles:  $\chi^2 = 0.661$ , 1 df, P = 0.42; genotypes:  $\chi^2 = 2.274$ , 2 df, P = 0.32), although the sample size (33 cases and 48 controls) had insufficient power to detect the subtle effect we were aiming to replicate. Thus we pooled the Cambridge and Edinburgh samples using a stratified analysis. This gave a significant test of association (P < 0.05), with estimated odds ratios of 0.72 (95% CI = 0.54-0.97) for allele B vs. allele A, and 0.55 (95% CI = 0.25-0.993) for genotype BB vs. genotype AA.

## Metaanalyses of the Tyrosine Hydroxylase Tetranucleotide Repeat Polymorphism

Three analyses were performed: bipolar (I and II) vs. control using allele frequencies from nine studies comprising 583 bipolar I and II patients and 745 controls; unipolar vs. control using results from three studies comprising 204 unipolar cases and 359 controls; and

bipolar + unipolar vs. control, using data from 10 studies comprising 846 cases and 823 controls.

The data from each study are summarized in Tables II (Cambridge data) and IV. The results of the metaanalyses (Table V) show that there was no significant association of TH tetranucleotide alleles with bipolar, unipolar, or bipolar + unipolar affective disorder groups.

## DISCUSSION

Several studies have implicated tyrosine hydroxylase in the pathogenesis of affective disorders. Initially, following linkage of Old Order Amish pedigrees with bipolar affective disorder to markers on chromosome 11p15.5, TH was proposed as a possible candidate [Egeland et al., 1987]. However, rediagnosis of key individuals and further investigation of additional members of the kindred failed to support the original study [Kelsoe et al., 1989]. In a French study, Leboyer et al. [1990] showed a positive association of bipolar affective disorder with the TH TaqI and BglII RFLPs, which are located 5' and 3' to coding sequences of the TH gene, respectively, a distance of approximately 9 kb [O'Malley et al., 1987; O'Malley and Rotwein, 1988]. In an extended study of 100 cases and 100 controls by the same group using the same RFLPs, Malafosse et al. [1997] found significantly different allele frequencies between the two groups. However, an association of bipolar affective disorder with these and other TH RFLPs has not been supported by other groups [Todd and O'Malley, 1989; Korner et al., 1990; Nothen et al., 1990; Gill et al., 1991; Inayama et al., 1993; Todd et al., 1996]. Meloni et al. [1995] reported, in their study of the TH tetranucleotide repeat polymorphism, that the BE genotype (corresponding to the 2,5 genotype of this study) was significantly overrepresented in their

TABLE IV. Data for Stratifed Analyses of Tyrosine Hydroxylase Tetranucleotide Repeat Polymorphism\*

Study	Author	Ethnic origin		Allele 1 (%)		Allele 3 (%)	Allele 4 (%)	Allele 5 (%)
2	Oruc et al., 1997	Croatian	Control	43 (31)	33(24)	13 (9)	14 (10)	37 (26)
			Bipolar	24(29)	16(19)	11(13)	12(14)	21(25)
			Unipolar	28(32)	15(17)	10 (11)	8 (9)	27(31)
			Bipolar + unipolar	52(30)	31(18)	21(12)	20(12)	48(28)
3	Souery et al., 1996b	Belgian	Control	45(34)	17(13)	16(12)	15(11)	41(31)
			Bipolar	34(26)	27(21)	17(13)	25(19)	29(22)
4	Meloni et al., 1995	French	Control	29(23)	18 (14)	19(15)	21(16)	41(32)
			Bipolar	33(26)	26(20)	9(7)	16(13)	44(34)
5	Perez de Castro et al., 1995	Spanish white	Control	20(21)	23(24)	18 (19)	11(11)	24(25)
			Bipolar	43 (36)	18(15)	12(10)	13(11)	34(28)
6	Korner et al., 1994 (Bonn)	German	Control	67(32)	28(13)	20(10)	43(21)	50(24)
			Bipolar	38 (30)	22(17)	23(18)	18 (14)	27(21)
7	Korner et al., 1994 (London)	British	Control	30 (29)	22(22)	9(7)	24(23)	17(17)
			Bipolar	31(29)	17(16)	14(13)	25(24)	19 (18)
8	Korner et al., 1994 (Cardiff)	British	Control	40 (37)	16(15)	12(11)	18(17)	22(20)
			Bipolar	46 (35)	21(16)	9 (9)	28(21)	28(21)
9	Souery et al., 1996a	Belgian	Control	37(37)	9 (9)	13(13)	8 (8)	35(35)
			Bipolar	38(27)	22(16)	17(12)	26(19)	37(26)
			Unipolar	22(31)	12(17)	8 (11)	11(16)	17(24)
			Bipolar + unipolar	60 (29)	34(16)	25(12)	37(18)	54(26)
10	Cavazzoni et al., 1996	Canadian Caucasian	Control	55(29)	35 (19)	26(14)	25(13)	47(25)
			Bipolar + unipolar	32(30)	22(20)	7(7)	25(23)	22(20)
11	Todd et al., 1996	North American white	Control	27	20	12	15	24
			Bipolar	37	12	14	14	20

\*Data for study 1 are shown in Table II.

French sample of bipolar patients when compared to controls. In the Cambridge data there was no such difference in this genotype in bipolar cases (8 out of 120) and controls (12 out of 239 genotypes). These results are similar to those reported by Todd et al. [1996] in their North American white population. In the other report of a positive association with the TH tetranucleotide repeat polymorphism, Perez de Castro et al. [1995] reported an excess of the th10 allele (allele 1) in their Spanish sample of bipolar cases vs. controls. Both studies were included in the metaanalysis of bipolar I + II cases and controls which comprised over 1,300 individuals. The absence of any association in this analysis suggests that this polymorphism is not in strong linkage disequilibrium with any putative functional variant which increases the risk of bipolar affective disorder.

Metaanalyses of combined bipolar and unipolar groups or the unipolar group alone also failed to demonstrate an association with the TH tetranucleotide repeat polymorphism. However, a weak association of the TH-*PstI* polymorphism with the unipolar sample was observed in the Cambridge sample, and this was maintained in the stratified analysis of Cambridge and Edinburgh samples. As our controls were unscreened, a proportion may have had sufficiently severe depression to be considered affected, resulting in a decrease in the power of our analysis. Since >97% of the controls are expected to be of UK Caucasian origin (see Materials and Methods), this study is unlikely to be confounded by population stratification.

In a German study of 33 unipolar and 99 controls using the BglII RFLP which is 3' to coding sequences, no significant differences were found [Korner et al., 1990], but Souery et al. [1996a] reported an excess of the 2-2 genotype in unipolar but not bipolar patients or controls using the 5' TH-TaqI RFLP in a Belgian study. The failure to detect an effect in the BglII study may be a function of its limited power, or of different degrees of linkage disequilibria with putative functional variants. Alternatively, the effect may be restricted to more se-

TABLE V. Stratified Analyses of Tyrosine Hydroxylase Tetranucleotide Repeat Polymorphism, Showing Odds Ratios With 95% Confidence Intervals (95% CI) for Each Allele\*

	$\chi^2$	df	P
Bipolar vs. control			
Test of association	1.16	4	>0.80
Test of heterogeneity between studies	46.10	32	0.051
Unipolar vs. control			
Test of association	3.17	4	>0.50
Test of heterogeneity between studies	9.29	8	0.32
Bipolar + unipolar vs. control			
Test of association	2.377	4	>0.50
Test of heterogeneity between studies	51.033	36	0.05

\*Odds ratios (OR) in all analyses are compared to allele 1. Bipolar vs. control (studies 1–8 and 11): for allele 2, OR = 1.04 (95% CI = 0.82–1.32); for allele 3, OR = 1.11 (95% CI = 0.85–1.45); for allele 4, OR = 1.11 (95% CI = 0.82–1.25). Unipolar vs control (studies 1, 2, and 9): for allele 2, OR = 0.86 (95% CI = 0.59–1.26); for allele 3, OR = 0.88 (95% CI = 0.57–1.34); for allele 4, OR = 0.99 (95% CI = 0.68–1.42); for allele 5, OR = 0.76 (95% CI = 0.55–1.05). Bipolar + unipolar vs. control (studies 1, 2, and 4–11): for allele 2, OR = 0.96 (95% CI = 0.78–1.19); for allele 3, OR = 0.95 (95% CI = 0.75–1.20); for allele 4, OR = 1.08 (95% CI = 0.88–1.33); for allele 5, OR = 0.92 (95% CI = 0.76–1.11).

vere recurrent unipolar cases, as defined by the inclusion criteria of the Cambridge sample. While the association of the TH-*Pst* I polymorphism with the unipolar sample does not remain significant after correcting for multiple testing, we believe that this finding needs to be replicated in a large powerful study.

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