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Analysis and Meta-Analysis of Two Serotonin Transporter Gene Polymorphisms in Bipolar and Unipolar Affective Disorders

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The serotonin transporter is a compelling candidate gene to examine in bipolar and unipolar affective disorder, since drugs that specifically inhibit the serotonin transporter can successfully treat depression. Previous association studies of a VNTR polymorphism in intron 2 and a functional insertion/deletion polymorphism in the promoter of this gene have produced conflicting results. The present study examined allele and genotype frequencies for both of these polymorphisms and resulting haplotypes in 87 English Caucasian bipolar patients, 125 English Caucasian unipolar affective disorder patients, and 174 controls. No significant associations were detected when these unipolar or bipolar cases were compared either separately or as a pooled "affective disorder" group to the controls. A meta-analysis of over 1,400 individuals of European Caucasian origin was then performed, comprising 772 controls, 375 bipolar and 299 unipolar patients for the VNTR polymorphism, and 739 controls, 392 bipolar and 275 unipolar patients for the promoter polymorphism. A significant association of promoter allele 2 was shown with bipolar (estimated odds ratio 1.21; 95% confidence interval 1.00–1.45), unipolar (OR 1.23; 95% CI 1.01–1.42), and combined bipolar + unipolar groups (OR 1.22; 95% CI 1.04–1.42). There was no demonstrable allelic association of the VNTR polymorphism with affective disorder: for the combined bipolar + unipolar group the odds ratios for VNTR alleles 9 and 10, compared with the common allele 12 were 1.05 (95% CI 0.56–1.95) and 0.90 (95% CI 0.77–1.05). These results suggest that the promoter allele 2, which has previously been shown to result in lower levels of serotonin transporter transcription, may be associated with affective disorder risk. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 81:58–63, 1998.

KEY WORDS: bipolar affective disorder; unipolar affective disorder; serotonin transporter polymorphisms

INTRODUCTION

Affective disorders are a common heterogeneous group of diseases characterized by disturbances of mood. The major affective disorders are usually divided into bipolar affective disorder (or manic-depression) and unipolar affective disorder. Twin, family, and adoption studies provide strong support for genetic factors in the pathogenesis of both disorders. In addition, some of these factors may overlap in the two diseases, since first-degree relatives of bipolar probands appear to have increased risks for both bipolar and unipolar affective disorders [McGuffin and Katz, 1989; Gershon, 1990; Nurnberger and Gershon, 1992].

The serotonin (or 5-hydroxytryptamine, 5-HT) transporter is a compelling candidate gene to examine in bipolar and unipolar affective disorder, since drugs that specifically inhibit the serotonin transporter can successfully treat depression. The human serotonin transporter gene is localised on chromosome 17q11-12 and encodes a transmembrane protein that functions in the re-uptake of 5-HT to the presynaptic neurons following neuro-release of 5-HT into the synaptic space

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[Ramamoorthy et al., 1993] as well as uptake of 5-HT to platelets [Lesch et al., 1993].

Two polymorphisms in the serotonin transporter gene have been examined in association studies of affective disorders. The first is a 3-allele VNTR in intron 2 [Ogilvie et al., 1995] and the second a 44 bp insertion/deletion within the promoter region, approximately 1 kb 5′ to the initiating methionine codon [Heils et al., 1996; Lesch et al., 1996]. The latter represents a functional variant, with the longer 528 bp allele being associated with increased transcription of the gene and higher biological activity of the transporter [Heils et al., 1996; Lesch et al., 1996; Collier et al., 1996b].

Studies of these polymorphisms have yielded confusing results. Ogilvie and colleagues found an association between the 9-repeat intronic VNTR allele and unipolar affective disorder risk in a small Scottish study [Ogilvie et al., 1995], whilst in two other studies in European or British Caucasians the risk was associated with the 12-repeat allele [Collier et al., 1996a; Craddock et al., 1996]. No associations of affective disorders with the intronic VNTR locus were found in studies of German [Stober et al., 1996] Japanese [Kunugi et al. 1996], or Chinese [Collier et al., 1996a] populations. In a combined European study involving three centres, Collier et al. [1996b] reported association of the (low biological activity) short promoter allele with a combined bipolar and unipolar affective disorder group. However, their stratified analysis failed to show a significant association with either bipolar or unipolar disorder, when these were considered as separate entities. Additionally, there was only modest linkage disequilibrium between the promoter polymorphism and the 12-repeat VNTR allele, which was associated with bipolar affective disorder irrespective of the nature of the promoter allele. Thus, they suggested that their results did not support the hypothesis that the association of the intronic VNTR was due to its linkage disequilibrium with the promoter polymorphism but that the effects of these two polymorphisms were independent and additive.

In order to clarify the relative importance of the serotonin transporter polymorphisms and affective disorder, we have performed an association study using English Caucasian samples of bipolar and unipolar affective patients. In addition, we conducted a meta-analysis of all the published data in order to evaluate more precisely the associations with the two polymorphisms.

MATERIALS AND METHODS

Patients

Eighty-seven unrelated patients (39 male, 48 female) with bipolar affective disorder were matched 1:2 with 174 unrelated controls for sex and age (within 24 months of the date of birth). One hundred and twenty-five patients with unipolar affective disorder (47 male, 78 female) were also included in the study. The bipolar and unipolar patients were recruited from in-patient and out-patient clinics in East Anglia and were English Caucasian in origin (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 age of onset of the first episode of depression between the ages of 18 and 65 years, at least two episodes of unipolar major depression, and at least one in-patient admission for the treatment of depression, in order to bias the sample towards a more severe disease phenotype. Control DNA from an anonymous 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 cases are Caucasian, about 80% have parents who were East Anglian and virtually all of the remainder are from the United Kingdom. Ethical approval for genetic studies of bipolar and unipolar affective disorder were obtained from local ethics committees.

Patients Included in Meta-Analyses

In addition to patients described above, the following were included in meta-analyses of the VNTR and promoter polymorphisms. The VNTR polymorphism included three independent studies: study 1, a Scottish study comprising 39 patients (20 male and 19 female) with single or recurrent major depressive episodes, 44 with bipolar disorder (23 male and 21 female) meeting DSM IV criteria, and 193 controls (125 male and 68 female) [Ogilvie et al., 1995]; study 2, 49 with recurrent unipolar depression, 53 with bipolar I disorder, all of German descent meeting DSM IV criteria, and 218 controls [Stober et al., 1996]; and study 3, 191 with bipolar affective disorder, 86 with unipolar depression of European Caucasian origin meeting DSM IV criteria, and 187 European Caucasian controls [Collier et al., 1996a]. The promoter polymorphism included three European groups: group 1, 191 with bipolar affective disorder (unrelated Caucasian of English residence), 86 with unipolar depression (unrelated Caucasian) meeting DSM IV criteria, and 187 unrelated Caucasian controls; group 2 were all of German Caucasian origin: 9 with bipolar I disorder, 47 with major depressive disorder meeting DSM IV criteria, and 301 controls; and group 3 were all of Italian Caucasian origin: 75 with bipolar I disorder, 22 with major depressive disorder meeting DSM IIIR criteria, and 95 controls [Collier et al., 1996b].

Polymerase Chain Reaction (PCR)

PCR across the serotonin transporter VNTR polymorphism in intron 2 was performed using the primers 5HTT-8224: 5′ gtc agt atc aca ggc tgc gag 3′ and 5HTT-8223: 5′ tgt tcc tag tct tac gcc agt g 3′ [Ogilvie et al., 1995]. Following an initial denaturation step at 94°C for 4.5 min, approximately 50–200 ng of DNA were amplified for 35 cycles at 94°C for 0.5 min, 61°C for 0.5 min, and 72°C for 0.5 min, followed by a final extension at 72°C for 10 min. The 20 μl reaction comprised 1 μl of DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 100 ng of each primer, 250 μM dNTPs, and 0.5 units *Taq* polymerase (GIBCO BRL). Products were analysed on

agarose gels (2% (w/v) agarose + 1% (w/v) NuSieve GT-G(FMC)) stained with ethidium bromide. Alleles were typed as 12, 11, or 9 repeats of the VNTR.

PCR across the serotonin transporter promoter insertion/deletion polymorphism was performed using the primers stpr5: 5′ ggc gtt gcc gct ctg aat gc 3′ and stpr3: 5′ gag gga ctg agc tgg aca acc ac 3′ [Heils et al., 1996]. Successful amplification was achieved using an initial denaturation step at 95°C for 4.5 min followed by 35 cycles of 95°C for 0.5 min, 61°C for 0.5 min, 72°C for 0.5 min, and a final extension at 72°C for 10 min. The 20 μl reaction comprised 1 μl of DNA (approximately 50–200 ng), 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 100 ng of each primer, 250 μM dNTPs (dGTP/7 deaza GTP 1:1), and 0.5 units *Taq* polymerase (GIBCO BRL). Products were analysed as above. The 528 bp and 484 bp products were typed as alleles 1 and 2, respectively.

The genotype frequencies of either polymorphism in the control group did not differ significantly from those expected from the allele frequencies, on the basis of Hardy-Weinberg equilibrium assumptions (VNTR polymorphism: $\chi^2 = 0.0069$, 5 degrees of freedom (df), $P>0.99$; promoter polymorphism: $\chi^2 = 0.00011$, 2 df, $P>0.99$).

Statistical Analysis

We used standard methodology for meta-analysis to combine data across studies. The pooled estimates of relative risk were estimated by using unconditional logistical regression, using the programme Splus. Overall tests of significance, confidence limits and heterogeneity tests were computed in the usual way [Breslow and Day, 1980].

RESULTS

Analysis of Cambridge Data

Bipolar and unipolar groups were compared to controls for the VNTR and promoter polymorphisms in the serotonin transporter gene. Following the precedent set in previous studies of this gene [Ogilvie et al., 1995; Collier et al., 1996; Stober et al., 1996; Kunugi et al. 1996], a combined bipolar and unipolar group was included in the analysis. Allele and genotype frequencies were not significantly different from the controls in either of the patient groups or in the combined unipolar and bipolar group (Table I, in all cases $P>0.05$).

Thirteen of eighteen possible combined serotonin transporter VNTR/promoter polymorphism genotypes were observed (results not shown). No significant differences were found when the groups were compared (Control vs. Bipolar $\chi^2 = 6.682$, 7 df, $P=0.46$; Control vs. Unipolar $\chi^2 = 5.552$, 7 df, $P=0.59$; and Control vs. Bipolar + Unipolar $\chi^2 = 6.540$, 7 df, $P=0.48$; analysis excludes rare genotypes: 10/10, 2/2; 12/9, 1/1; 12/9, 1/2; 10/9, 1/2; and 9/9, 1/2). Serotonin transporter phase-known haplotypes were determined where possible (i.e., in individuals that were homozygous for one or both loci), which represented 72%, 80%, 72%, and 75% of the observed genotypes of control, bipolar, unipolar, and combined bipolar + unipolar groups, respectively. Comparison of phase-known haplotypes in these groups showed that there were no significant differences between any of the affective disorder patient groups and the controls (Control vs. Bipolar $\chi^2 = 0.941$, 3 df, $P=0.816$; Control vs. Unipolar $\chi^2 = 4.921$, 3 df, $P=0.18$; and Control vs. Bipolar + Unipolar $\chi^2 = 3.068$, 3 df, $P=0.381$; analysis excludes rare allele-9 bearing haplotypes). Comparison of phase-known hap-

TABLE I. Genotype and Allele Frequencies for Serotonin Transporter VNTR and Promoter Polymorphisms*

	Control	Bipolar	Unipolar	Bipolar + unipolar
VNTR genotypes				
12 12	58 (33.3%)	37 (42.5%)	43 (34.4%)	80 (37.7%)
12 10	81 (46.6%)	29 (33.3%)	48 (38.4%)	77 (36.3%)
10 10	27 (15.5%)	16 (18.4%)	29 (23.2%)	45 (21.2%)
12 9	6 (3.4%)	4 (4.6%)	5 (4.0%)	9 (4.2%)
10 9	0 (0.0%)	1 (1.1%)	0 (0.0%)	1 (0.5%)
9 9	2 (1.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	174	87	125	212
VNTR alleles				
12	203 (58.3%)	107 (61.5%)	139 (55.6%)	246 (58.0%)
10	135 (38.8%)	62 (35.6%)	106 (42.4%)	168 (39.6%)
9	10 (2.9%)	5 (2.9%)	5 (2.0%)	10 (2.4%)
Total	348	174	250	424
Promotor genotypes				
1 1	59 (34.9%)	31 (35.6%)	35 (28.0%)	66 (31.1%)
1 2	81 (47.9%)	36 (41.4%)	64 (51.2%)	100 (47.2%)
2 2	29 (17.2%)	20 (23.0%)	26 (20.8%)	46 (21.7%)
Total	169	87	125	212
Promoter alleles				
1	199 (58.9%)	98 (56.3%)	134 (53.6%)	232 (54.7%)
2	139 (41.1%)	76 (43.6%)	116 (46.4%)	192 (45.3%)
Total	338	174	250	424

*Allele and genotype frequencies were compared in the controls to the unipolar, bipolar and unipolar + bipolar groups using χ^2 tests. For comparison of the VNTR non-significant genotypes, we excluded 10/9 and 9/9, since the numbers in each cell were too small. All comparisons were nonsignificant at the 5% level.

lotypes of common alleles in the control group to the haplotype distribution expected if there was random assortment of haplotypes at these two loci revealed a modest degree of linkage disequilibrium ($\chi^2 = 14.638$, 3 df, $P<0.01$), in agreement with the conclusions of Collier et al. [1996b].

Meta-Analysis

In view of the conflicting data concerning the association of affective disorder with either polymorphism, a meta-analysis was performed that included data from the present study together with published data from other groups. The meta-analysis of the VNTR polymorphism included the studies of Ogilvie et al. [1995], Stober et al. [1996], and Collier et al. [1996a]; the Japanese study [Kunugi et al., 1996] was excluded because this population appears to have completely different normal allele distributions at this locus. The promoter analysis included the study of Collier et al. [1996b], which reported results from three European centres (London, Milan, and Wurzburg). This meta-analysis (Table II) examined 772 controls, 375 bipolar and 299 unipolar patients for the VNTR polymorphism, and 739 controls, 392 bipolar and 275 unipolar patients for the promoter polymorphism, all of European Caucasian origin. We found no significant allelic association of the VNTR polymorphism with affective disorder, but a significant association of promoter allele 2 with disease risk in the bipolar (odds ratio 1.21; 95% Confidence Intervals 1.00–1.45; $\chi^2 = 3.86$, 1 df, $P = 0.049$), unipolar (odds ratio 1.23; 95% CI 1.01–1.52; $\chi^2 = 4.10$, 1 df, $P = 0.042$) and the combined bipolar + unipolar groups (odds ratio 1.22; 95% CI 1.04–1.42; $\chi^2 = 6.00$, 1 df, $P = 0.014$). In addition, homozygotes for the 2/2 promoter allele were at increased risk for disease in the combined unipolar + bipolar group (odds ratio 1.50; 95% CI 1.09–2.05; $\chi^2 = 6.73$, 2 df, $P = 0.035$). There was no evidence of heterogeneity in any of the odds ratio estimates between the studies.

DISCUSSION

Previous studies have suggested that two different polymorphisms in the serotonin transporter gene are associated with risk for affective disorder [Ogilvie et al., 1995; Collier et al., 1996a,b; Craddock et al., 1996]. The association of the VNTR polymorphism has not been consistently replicated [present study, Stober et al., 1996; Kunugi et al. 1996] and different high-risk alleles have been reported by different groups on UK populations [Ogilvie et al., 1995; Collier et al., 1996a; Craddock et al., 1996]. It is also not clear whether the intronic VNTR shows an association with disease merely because of a weak linkage disequilibrium with the functional promoter variant or whether it is associated with an independent effect.

Our local study on its own and all previous single centre analyses [Collier et al., 1996b] failed to reveal any significant associations with the serotonin transporter promoter polymorphism. Since our controls were unscreened, a proportion may have had sufficiently severe depression to be considered affected. This would have decreased the power of our analysis. In their stratified analysis, Collier et al. [1996b] found a significant association with a combined bipolar and unipolar group but not with either bipolar or unipolar groups when considered separately. Our meta-analysis, which includes published data, does suggest some association between the serotonin transporter functional promoter polymorphism and risk for both bipolar and unipolar disease. Although the association was only just significant when bipolar disease alone was considered as the phenotype, the consistency between the odds ratios in bipolar and unipolar disease provides further support for this association. The overall association was fairly weak with an estimated odds ratio of 1.22 for allele 2 (decreased serotonin transporter transcription [Heils et al., 1996; Lesch et al., 1996; Collier et al., 1996b]) or 1.50 for the 2/2 homozygotes, compared with the 1/1 homozygotes for the combined bipolar and unipolar group. It is possible that there may have been some lack of uniformity in the diagnoses of the different centres. This may have led to an underestimation of the odds ratios.

These small odds ratios will be difficult to detect with the sample sizes used in most single-centre association studies and justify the meta-analysis approach. For example, our sample of 87 bipolar cases and 169 controls would only have an 80% power to detect a relative risk of 2.0 (5% significance), but with the meta-analysis sample of 392 bipolar cases and 739 controls it is possible to detect a relative risk of <1.5 with the same power and degree of significance [Breslow and Day 1980]. Although the promoter alleles result in differences in serotonin transporter transcription, these studies do not provide formal proof of a causal role in affective disorders. While these associations are unlikely to be due to another functional variant in linkage disequilibrium with the promoter polymorphism, this possibility cannot be discounted. Recently, promoter allele 2 was also associated with anxiety-related personality traits [Lesch et al., 1996], suggesting that variation in serotonin transporter activity may be associated with diverse phenotypes.

The meta-analysis of 772 controls, 375 bipolar, and 299 unipolar patients for the VNTR polymorphism failed to reveal any significant associations and the 95% confidence intervals suggest that this polymorphism is unlikely to have a major influence of disease risk.

These data are consistent with previous reports of decreased serotonin transporter levels in brains of depressed and suicide patients that were estimated by using radiolabeled ligands for the transporter, either [^{3}H] imipramine, or the more selective uptake inhibitor [^{3}H] citalopram [Stanley et al., 1982; Perry et al., 1983; Leake et al., 1991]. Intuitively, one would expect decreased serotonin transporter activity to be associated with raised inter-synaptic 5-HT levels in patients with bipolar and unipolar affective disorder. However depression has been associated with reduced levels of this neurotransmitter and specific inhibitors of 5-HT uptake are effective in treating depression. Routledge and Middlemiss [1996] have addressed this paradox by arguing that the lower activity of the "2" allele promoter variant may have consequences similar to those ob-

TABLE II. Meta-Analysis of Allele Frequencies and Genotypes of Serotonin Transporter VNTR and Promoter Polymorphisms*

		Allele	Control	Bipolar	Unipolar	Bipolar + unipolar
VNTR alleles						
Ogilvie *et al.*	Scottish	12	229 (59.3%)	46 (52.3%)	44 (56.4%)	90 (54.2%)
		10	153 (39.6%)	40 (45.4%)	29 (37.2%)	69 (41.6%)
		9	4 (1.0%)	2 (2.3%)	5 (6.4%)	7 (4.2%)
Stober *et al.*	German	12	237 (54.4%)	54 (50.9%)	56 (57.1%)	110 (53.9%)
		10	193 (44.3%)	52 (49.1%)	41 (41.8%)	93 (45.6%)
		9	6 (1.4%)	0 (0.0%)	1 (1.0%)	1 (0.5%)
Collier *et al.*	European	12	202 (54.0%)	259 (67.8%)	96 (55.8%)	355 (64.1%)
	Caucasian	10	169 (45.2%)	120 (31.4%)	76 (44.2%)	196 (35.4%)
		9	3 (0.8%)	2 (0.8%)	0 (0.0%)	3 (0.5%)
		Allele	Control	Bipolar	Unipolar	Bipolar + unipolar
Promoter alleles						
Collier *et al.*						
London	European	1	193 (55.5%)	189 (49.7%)	80 (49.4%)	269 (49.6%)
	Caucasian	2	155 (44.5%)	191 (51.3%)	82 (51.6%)	273 (51.4%)
Milan	Italian	1	108 (56.8%)	84 (56.0%)	22 (50.0%)	106 (54.6%)
		2	82 (43.2%)	66 (44.0%)	22 (50.0%)	88 (45.4%)
Wurzburg	German	1	359 (59.6%)	39 (50.0%)	53 (56.4%)	92 (53.5%)
		2	243 (40.4%)	39 (50.0%)	41 (43.6%)	80 (46.5%)
		Genotype	Control	Bipolar	Unipolar	Bipolar + unipolar
Promoter genotypes						
Collier *et al.*						
London	European	1 1	55 (31.6%)	49 (25.7%)	23 (28.4%)	72 (26.6%)
	Caucasian	1 2	83 (47.7%)	91 (47.6%)	34 (42.0%)	125 (46.1%)
		2 2	36 (20.7%)	51 (26.7%)	24 (29.6%)	75 (27.3%)
Milan	Italian	1 1	28 (29.5%)	25 (33.3%)	7 (31.8%)	32 (33.0%)
		1 2	52 (54.7%)	34 (45.3%)	8 (36.4%)	42 (43.3%)
		2 2	15 (15.8%)	16 (21.3%)	7 (31.8%)	23 (23.7%)
Wurzburg	German	1 1	108 (35.9%)	8 (20.5%)	16 (34.0%)	24 (27.9%)
		1 2	143 (47.5%)	23 (59.0%)	21 (44.7%)	44 (51.2%)
		2 2	50 (16.6%)	8 (20.5%)	10 (21.3%)	18 (20.9%)

Odds ratios for meta-analysis data shown above

VNTR Alleles: Allele 10 or allele 9 vs. allele 12

	Allele 10 odds ratio (95% CI)	Allele 9 odds ratio (95% CI)	Test of association		Test of heterogeneity between studies	
			χ^2: 2 df	*P*	χ^2: 6 df	*P*
Bipolar	0.82 (0.68–0.99)	0.88 (0.41–1.91)	4.29	0.12	16.72	NS
Unipolar	1.01 (0.83–1.23)	1.16 (0.55–2.50)	0.16	0.92	10.76	NS
Bipolar + unipolar	0.90 (0.77–1.05)	1.05 (0.56–1.95)	2.92	0.23	14.96	NS

Promoter alleles

	2 vs. 1 odds ratio (95% CI)	Test of association		Test of heterogeneity between studies	
		χ^2: 1 df	*P*	χ^2: 3 df	*P*
Bipolar	1.21 (1.00–1.45)	3.86	0.049	4.84	NS
Unipolar	1.23 (1.01–1.52)	4.10	0.042	0.19	NS
Bipolar + unipolar	1.22 (1.04–1.42)	6.00	0.014	0.48	NS

Promoter genotypes

	1 2 vs. 1 1 odds ratio (95% CI)	2 2 vs. 1 1 odds ratio (95% CI)	Test of association		Test of heterogeneity between studies	
			χ^2: 2 df	*P*	χ^2: 6 df	*P*
Bipolar	1.09 (0.81–1.48)	1.48 (1.02–1.53)	4.56	0.100	5.06	NS
Unipolar	1.06 (0.76–1.49)	1.55 (1.04–2.32)	5.15	0.076	3.02	NS
Bipolar + unipolar	1.10 (0.85–1.41)	1.50 (1.09–2.05)	6.73	**0.035**	2.77	NS

*Data are analysed together with data presented in Table 1.

[a]χ^2 and *P* values are shown for the allele and genotype comparisons in cases and controls and for the analysis of the heterogeneity between studies. *P* values < 0.05 are shown in bold face.

served after acute treatment with specific serotonin reuptake inhibitors, where there are increases in 5-HT near the cell bodies and dendrites in the midbrain raphe nuclei, but no changes or reductions in terminal 5-HT levels. These effects are thought to be mediated by negative feedback on serotonergic neuronal activity via somatodendritic 5-HT_{1A} receptors at the raphe nuclei. Thus, this negative feedback could reconcile the low serotonin transporter function of the "2 allele" variant with the lower terminal 5-HT output associated with depression.

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NOTE ADDED IN PROOF

Recently Battersby *et al.* (Psychiatric Genetics 1996, 6:177–181) reported an association in a Scottish population of the 5HTT VNTR allele 9 with affective disorder in a sample which included the cases reported by Ogilvie *et al.* (1996). Replacement of the 5HTT VNTR data of Ogilvie *et al.* with those of Battersby *et al.* in our metaanalysis showed no significant association of either allele 10 vs 12 or allele 9 vs allele 12 of the VNTR polymorphism with bipolar, unipolar, or bipolar + unipolar affective disorder (in all cases $p > 0.05$).

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