Molecular Psychiatry (1998) 3, 186–189

GUEST EDITORIAL

Serotonin transporter candidate gene studies in affective disorders and personality: promises and potential pitfalls

While affective and anxiety disorders are substantially heritable, their inheritances are complex and multifactorial.[1] It is therefore unsurprising that such etiologically heterogeneous illnesses have not easily succumbed to genetic methodologies that are further challenged by an imperfect classification system.[2] Nevertheless, investigators continue to behave as if the problems are not insoluble. Association studies of candidate genes have lately held particular appeal, at least partly since this approach connects well with the familiar view that these disorders arise from alterations in particular brain neurochemical systems.

The evidence implicating brain serotonin (5-HT) neurotransmission in anxiety and affective disorders is extensive and compelling.[3,4] In the attempt to investigate whether alterations in function of brain 5-HT systems are etiologically associated with these illnesses, attention has focused on polymorphisms in genes encoding components of the serotonergic system. These genes are seen as potential candidates influencing susceptibility, course, or symptoms. Researchers are investigating variants of genes for the 5-HT synthesis pathway, the 5-HT transporter (5-HTT), and several of the numerous 5-HT receptors. Potentially important sequence variations may occur in the coding region of a gene, in gene promoters (which could affect transcription efficiency), and in intronic regions, which, while non-coding, might affect gene expression or distribution of the gene product. Some variants of serotonin system genes are common, consistent with a role in complex, polygenic disorders with relatively high population incidences.

The most common research strategy used in association studies, because it is often logistically easiest, is the population-based approach. Such studies compare the frequencies of polymorphic alleles in a group of individuals with a particular psychiatric diagnosis with those in a reference population. The use of this method is now widespread. Although this strategy has important limitations (see below), initial findings have been intriguing enough to spur further research by diverse groups of investigators.

A number of reports have described associations between affective disorders and polymorphisms in or near the 5-HTT gene. The 5-HTT is a major modulator of serotonergic neurotransmission in brain regions crucial to emotional behavior and is the target of the most widely used class of antidepressant and antianxiety agents, the serotonin reuptake inhibitors. It is therefore a highly logical candidate for involvement in affective disorders. While no common coding sequence variants have been found in patients with depression or one anxiety disorder,[5,6] common polymorphisms in the intronic and promoter regions of the gene have been identified.[7,8]

An early report found an association between a variable number tandem repeat (VNTR) in intron 2 of the 5-HTT gene and affective illness in a small sample of Scottish patients *vs* controls (most were anonymous blood donors). In that study, an excess of the rare 9-repeat allele was found in the patients with unipolar affective illness compared to the control sample.[8] However, a number of other studies, using larger samples from a number of countries, have failed to find an association between the 9-repeat allele and affective illness.[10–13]

Two population-based studies recently published in this journal[13,14] also failed to find an association with the 9-repeat allele but argue instead for an association between bipolar disorder and another 5-HTT VNTR allele, characterized by 12 repeats. An association between bipolar disorder and the 12-repeat allele was first reported by Collier *et al*[10] in a study of cases and controls from the UK. This association was not found in the earlier UK study,[8] or in patient samples from Germany[11] or France.[12] In each of the three positive reports, the 12-repeat allele was by far the most common allele in both cases and controls, and the association with bipolar disorder, while significant, was of relatively small magnitude, with an odds ratio of 1.2–3.1 for persons carrying one or two 12-repeat alleles. By comparison, consider that the odds ratios are 170 for HLA-DR2 and narcolepsy[15] or 2.7–11.2 for ApoE4 and Alzheimer disease.[16]

All these studies used the population-based association method in which cases and controls are unrelated, although (hopefully) comparable. A major problem inherent in these population-based association studies is that significant-appearing relationships may be found as an artifact of genetic differences between the cases and controls unrelated to the phenotype of interest. Such artifacts may occur, for example, if the affected and control populations are ethnically different. However, even when investigators try to control for ethnic differences, another artifact, called 'admix-

Correspondence: BD Greenberg, MD, PhD, Chief, Adult OCD Unit, NIMH, NIH Clinical Center, Bldg 10/3D-41, Bethesda, MD 20892-1264, USA. E-mail: bdg@helix.nih.gov
Received 24 March 1998; accepted 25 March 1998

ture' by population geneticists, remains a potential pitfall. Admixture applies whenever the population under study has not undergone random mating for at least a few generations. Thus even cases and controls that appear well-matched ethnically may differ in ways that can produce strong—but nonetheless spurious—allelic association results.[17] Family-based studies, which compare cases with relatives (usually parents), control for admixture, for potential differences in polymorphism frequencies across ethnic groups, as well as for other population biases.[18,19] Obtaining a family sample requires collecting cases and at least one parent, and can be more difficult and time-consuming than collecting a comparably-sized sample of unrelated cases and controls. Still, the risk of spurious associations arising from stratification and admixture implies that population-based association reports—while a useful starting point—should be viewed as preliminary until confirmed by family-based association studies.

How then to interpret the three recent population-based association studies of the 5-HTT intron 2 VNTR which found an association of the 12-repeat allele with affective disorder, and the four which did not? The first issue is whether the studies that followed the original report of association by Collier *et al*,[10] had adequate statistical power to either replicate or reject that finding. Some 205 cases with bipolar disorder would be needed to achieve 80% power to replicate the Collier *et al*[10] finding, assuming the modest effect size (odds ratio of 1.8) and frequencies of the 12-repeat allele seen in the initial report (0.63 in cases and 0.56 in controls), and an alpha of 0.05 (InStat 2.01, GraphPad Inc, San Diego, CA, USA). By this calculation, only Rees *et al*[13] and Kunugi *et al*[14] would have what is generally considered to be adequate statistical power to replicate the initial finding in bipolar disorder.[10] This illustrates the difficulty of replicating small effects of genetic variants, even if such influences ultimately prove biologically meaningful.

Given the potential problem of inadequate statistical power in individual studies, another way to assess the potential role of the intron 2 VNTR is to do an overall chi-square analysis. Performing a combined chi-square, however, immediately raises other important methodological issues. One of the studies supporting an association (Kunugi *et al*)[14] cannot easily be considered with the rest, since the allele frequencies obtained in that study of Japanese patients and controls diverge substantially from those in the remaining studies of mainly European samples. This ethnic heterogeneity argues for excluding the data from this non-comparable sample from the analysis.

An overall chi-square, using published data, is presented in Table 1. Without the data from Kunugi *et al*,[14] the results of an overall chi-square which includes the original Collier *et al*[10] study support association of bipolar illness with the 12-repeat 5-HTT intron 2 VNTR allele. However, without the data from the original study, a chi-square combining the remaining data, despite a relatively greater frequency of allele 12 in the bipolar probands compared to controls (of 0.61 in cases and 0.57 in controls, respectively), fails to find a significant association, even when one-tailed *P*-values are used (see Table 1).

Where does this leave us? Bearing in mind that even the total sample size used in the combined analysis performed may be inadequate to replicate even an actual association of such small magnitude,[20] genetic variation at or near the 5-HTT gene may hold promise as a candidate locus in affective disorders—albeit as a gene of small effect. There is a compelling need to add family-based association, linkage, and mutation detection studies to the findings available thus far. One linkage study of the 5-HTT region was inconclusive,[21] and a systematic screen of the whole coding sequence in patients with affective disorder found a sequence variant in only a single patient.[22] As Hill pointed out in his classic paper on the subject of case-control studies in medicine, consistency, specificity, and coherence with results of other approaches are hallmarks of valid associations.[23]

An additional difficulty, particularly in studying intronic polymorphisms in candidate genes, is that their functional significance may not be established. While highly polymorphic markers very tightly linked to a functional polymorphism are often useful in this research,[24,25] studying candidate polymorphisms that are shown to directly affect gene function can be a more powerful strategy. One such variant is a common polymorphism in the promoter region of the 5-HTT gene. A variable insertion-deletion in a repeat element segment ~1 kb upstream of the 5-HTT promoter (the 5-HTT-linked promoter region or 5-HTTLPR) was found to affect transcription of the 5-HTT gene in an *in vitro* human cell assay.[7] The short promoter region allele was found to have a small but significant association with affective illness in a large population-based case-control study in which most of the cases had bipolar illness.[26] However, an association with affective illness was not found in two other studies with smaller numbers of affectively ill patients and controls.[12,13] No family-based association studies of this polymorphism in patients with affective disorders have to our knowledge been published. We have performed a preliminary analysis of this polymorphism in a family-based association study of 51 probands with bipolar I affective disorder (manic-depressive illness) and their parents. We found no evidence of association using the transmission disequilibrium test and a nonsignificant trend towards association of the long 5-HTTLPR allele with bipolar disorder using the haplotype relative risk test (Greenberg, McMahon, Bengel, and Murphy, unpublished observations). This finding contrasts with that of Collier *et al*[26] who found a small but significant association between the short allele and affective illness in their population-based study. Taken together, these results suggest that the putative 5-HTTLPR association is either spurious or the result of linkage disequilibrium between the 5-HTTLPR and another functionally significant nucleotide variant at the 5-HTT locus.

Another approach to association studies takes advan-

Table 1 Association studies of the 5-HTT intron 2 VNTR polymorphism and affective illness

Study		*Allele 9*	*Allele 10*	*Allele 12*	χ^2	*P*	*Sample*
Battersby *et al*[35]	UP	10	101	147	8.27	0.01	Scottish (includes
	BP	8	87	141	6.73	0.0692	Ogilvie *et al*[8])
	TOT	18	188	288	9.85	0.007	
	C	7	279	406	–		
Stober *et al*[11]	UP	1	33	40	0.18	ns	German
	BP	0	42	43	1.86	ns	
	TOT	1	75	83	1.26	ns	
	C	6	141	160	–		
Collier *et al*[10]	UP	0	76	96	1.47	ns	UK
	BP	3	120	259	15.27	0.001	(Caucasian
	TOT	3	196	355	9.47	0.018	subset)
	C	3	169	202	–		
Bellivier *et al*[12]	BP	1	83	134	0.031	ns	French
	C	1	65	106	–		
Rees *et al*[13]	UP	5	57	70	1.09	ns	UK
	BP	5	120	217	3.48	0.031	
	TOT	10	177	287	1.57	(1-tailed)	
	C	5	102	135			
Overall χ^2 (A) with	BP	17	452	794	13.15	0.0028	
Collier *et al*[10]	C	22	756	1009	–		
Overall χ^2 (B) minus	BP	14	332	535	3.45	0.16	
Collier *et al*[10]	C	19	587	807	–	(1-tailed)	

UP = unipolar affective disorder; BP = bipolar affective disorder; TOT = total affective disorder sample; C = control sample; *P*-values are 2-tailed unless noted; the *P*-value in Rees *et al*[13] was given as 1-tailed, testing the prior report of association (Collier *et al*).[10]

tage of well-characterized individual differences in human behavior along particular dimensions. Such traits, conceptualized in somewhat different but related ways in different models of temperament or personality, are relatively enduring and substantially heritable.[27] A human quantitative trait particularly important to mood and anxiety disorders is 'neuroticism', the tendency towards negative emotional experiences, particularly anxiety and depression. Higher levels of neuroticism are found in patients with anxiety and affective disorders. In a study by Lesch and colleagues, individuals with the short form of the 5-HTTLPR polymorphism had a small but significant increase in neuroticism scores, accounting for 3–4% of the overall variance.[9] This association was found both across and within families in a mostly male general population sample of over 500 individuals.

Two subsequent studies, using smaller samples (of 106 and 120 individuals, respectively) and population-based designs, found no significant association between 5-HTTLPR genotype and neuroticism[28] or the related trait of harm avoidance.[29] However, the sample size in both studies was likely insufficient to detect association. Moreover, one of the studies[28] selected individuals on the basis of extreme neuroticism scores, an approach which relies on the assumption that 5-HTTLPR genotype affects neuroticism uniformly across the distribution. A re-analysis of our original sample[9] prompted by these findings, found that the contribution of the 5-HTTLPR to neuroticism was actually greatest in the middle of the distribution and nonsignificant at the extremes (D Hamer, personal communication), illustrating the desirability of obtaining genotypes from individuals across the distribution of a continuous trait. It is likely that the method of extremes will be useful in some instances and potentially misleading in others. Two recent studies showing a lack of association between 5-HTT genotype and panic disorder, an illness characterized by high levels of neuroticism and marked anxiety-proneness, are consistent with the idea that, in a general population sample, genetic influences may differ between individuals at the extremes and those across the overall range of a personality dimension.[30,31]

More definitive studies investigating the association between neuroticism, anxiety, depression and the 5-HTTLPR await larger samples and family-based designs. An example of the latter is the observation of significant positive linkage between the 5-HTTLPR and harm avoidance subscales in a large sample of Finnish sib-pairs,[32] which provides converging evidence that the 5-HTTLPR may influence personality traits related to neuroticism. It is to be hoped that studies such as these will advance our understanding not only of possible genetic influences on personality, but will also be useful in refining conceptions of the relevant personality traits themselves, the disorders associated with them, and possible approaches to the treatment of those disorders.

Finally, it needs to be emphasized that association studies focusing only on one neurotransmitter system, even as attractive a candidate as the serotonergic sys-

tem, are unlikely to tell the whole story.[33] It may be most informative, as candidate gene studies go forward, for investigators to test polymorphisms which are known to be functionally significant. Larger samples, with statistically adequate power to distinguish meaningful replications and non-replications, and family-based designs will be needed. Linkage and association strategies are likely to become increasingly complementary approaches to studying complex diseases and behavioral dimensions.[32,34] Findings using newer methods in development that combine the categorical and dimensional approaches, or other techniques that account for the susceptibility enhancing (and reducing) influences of multiple genes, are needed. Nevertheless, the relatively high density of intriguing 5-HTT association findings over a short time is grounds for cautious optimism, together with the expectation of a long and often frustrating road ahead.

BD Greenberg[1], FJ McMahon[2] and DL Murphy[1]
[1]Laboratory of Clinical Science
National Institute of Mental Health;
[2]Department of Psychiatry and Behavioral Sciences,
The Johns Hopkins University School of Medicine

References

1 Gershon ES, Cloninger CR (eds). *Genetic Approaches to Mental Illness*. APA Press: Washington, DC, 1994, pp 3–106.
2 Philibert RA, Egeland JA, Paul SM, Ginns EI. The inheritance of bipolar affective disorder: abundant genes coming together. *J Affective Disord* 1997; **43**: 1–3.
3 Heninger GR. The role of serotonin in clinical disorders. In: Bloom F, Kupfer D (eds). *Psychopharmacology: The Fourth Generation of Progress*. Raven Press: New York, 1995, pp 471–482.
4 Greenberg BD, Altemus M, Murphy DL. The role of neurotransmitters and neurohormones in obsessive-compulsive disorder. *Int Rev Psychiatry* 1997; **9**: 31–44.
5 Altemus M, Murphy DL, Greenberg B, Lesch KP. Intact coding region of the serotonin transporter gene in obsessive-compulsive disorder. *Am J Med Genet* 1996; **67**: 409–411.
6 Lesch K-P, Franzek E, Gross J, Wolozin BL, Riederer P, Murphy DL. Primary structure of the serotonin transporter in unipolar and bipollar disorder. *Biol Psychiatry* 1995; **37**: 215–223.
7 Heils A, Teufel A, Petri S, Seemann M, Bengel D, Balling U *et al*. Functional promoter and polyadenylation site mapping of the human serotonin (5-HT) transporter gene. *J Neural Transm* 1995; **102**: 247–254.
8 Ogilvie AD, Battersby B, Bubb VJ, Fink G, Harmar AJ, Goodwin GM *et al*. Polymorphism in serotonin transporter gene associated with susceptibility to major depression. *Lancet* 1996; **347**: 731–733.
9 Lesch K-P, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S *et al*. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527–1531.
10 Collier DA, Arranz MJ, Sham P, Battersby S, Gill P, Aitchison KJ *et al*. A VNTR in the serotonin transporter gene is a potential susceptibility factor for bipolar affective disorder. *NeuroReport* 1996; **7**: 1675–1679.
11 Stober G, Heils A, Lesch K-P. Serotonin transporter gene polymorphism and affective disorder. *Lancet* 1996; **347**: 1340–1341.
12 Bellivier F, Laplanche J-L, Leboyer M, Feingold J, Bottos C, Allilaire J-F *et al*. Serotonin transporter gene and manic depressive illness: an association study. *Biol Psychiatry* 1997; **41**: 750–752.
13 Rees M, Norton N, Jones I, McCandless F, Scourfield J, Holmans P *et al*. Association studies of bipolar disorder at the human serotonin transporter gene (hSERT; 5HTT). *Mol Psychiatry* 1997; **2**: 398–402.
14 Kunugi H, Hattori M, Kato T, Tatsumi M, Sakai T, Sasaki T *et al*. Serotonin transporter gene polymorphisms: ethnic difference and possible association with bipolar affective disorder. *Mol Psychiatry* 1997; **2**: 457–462.
15 Brown MA, Pile KD, Kennedy LG, Calin A, Darke C, Bell J *et al*. HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. *Ann Rheumatic Dis* 1996; **55**: 268–270.
16 Bickeboller H, Compion D, Brice A. Apolipoprotein E and Alzheimer disease. *Am J Hum Genet* 1997; **60**: 439–446.
17 Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet* 1996; **59**: 983–989.
18 Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus. *Am J Hum Genet* 1993; **52**: 506–516.
19 Thomson G. Mapping disease genes: family-based association studies. *Am J Hum Genet* 1995; **57**: 487–498.
20 Suarez BK, Hampe CL, O'Rourke D, Van Eerdewegh P, Reich T. Sib-based detection of QTLs. *Genet Epidemiol* 1995; **12**: 675–680.
21 Kelsoe J, Remick R, Sadovnick D *et al*. Genetic linkage study of bipolar disorder and the serotonin transporter. *Am J Med Genet* 1996; **67**: 215–227.
22 Di Bella D, Catalano M, Balling U, Smeraldi E, Lesch K-P. Systematic screening for mutations in the coding region of the human serotonin transporter (5-HTT) gene using PCR and DGGE. *Am J Med Genet* 1996; **67**: 541–545.
23 Hill AB. The environment and disease: association or causation? *Proc Royal Soc Med* 1965; **58**: 295–300.
24 Tsiouris SJ, Breschel TS, Xu J, McInnis MG, McMahon FJ. Linkage disequilibrium analysis of G-olf alpha (GNAL) in bipolar affective disorder. *Am J Med Genet* 1996; **67**: 491–494.
25 Bennett ST, Todd JA. Human type 1 diabetes and the insulin gene: principles of mapping polygenes. *Ann Rev Genet* 1996; **30**: 343–370.
26 Collier DA, Stober G, Li T, Heils A, Catalano M, Di Bella D *et al*. A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. *Mol Psychiatry* 1996; **1**: 453–460.
27 Plomin R, Owen MJ, McGuffin P. The genetic basis of complex human behaviors. *Science* 1994; **264**: 1733–1739.
28 Ball D, Hill L, Freeman B, Eley TC, Strelau J, Riemann R *et al*. The serotonin transporter gene and peer-rated neuroticism. *NeuroReport* 1997; **8** 1301–1304.
29 Ebstein RP, Gritsenko I, Nemanov L, Frisch A, Osher Y, Belmaker RH. No association between the serotonin transporter gene regulatory region polymorphism and the tridimensional personality questionnaire (TPQ) temperament of harm avoidance. *Mol Psychiatry* 1997; **2**: 224–226.
30 Deckert J, Catalano M, Heils A, Di Bella D, Friess F, Politi E *et al*. Functional promoter polymorphism of the human serotonin transporter: lack of association with panic disorder. *Psychiatric Genet* 1997; **7**: 45–47.
31 Matshushita S, Muramatsu T, Kimura M, Shirakawa O, Mita T, Nakai *et al*. Serotonin transporter gene regulatory region polymorphism and panic disorder. *Mol Psychiatry* 1997; **2**: 390–392.
32 Mazzanti CM, Lappalainen J, Long JC, Naukkarinen H, Eggert M, Virkkunen M *et al*. Role of the serotonin transporter promoter polymorphism in anxiety related traits. *Arch Gen Psychiatry* (in press).
33 Boyer WF, Feighner JP. The serotonin hypothesis: necessary but not sufficient. In: Feighner and Boyer (eds). *Selective Serotonin Reuptake Inhibitors*. John Wiley & Sons: Chichester, 1991, pp 71–80.
34 Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996; **273**: 1516–1517.

Reference added in proof

35 Battersby S, Ogilvie AD, Smith CAD, Blackwood DHR, Muir WJ, Quinn WJ *et al*. Structure of a variable number tandem repeat of the serotonin transporter gene and association with affective disorder. *Psychiat Genet* 1996; **6**: 177–181.