ORIGINAL RESEARCH ARTICLE

Low activity allele of catechol-O-methyltransferase gene associated with rapid cycling bipolar disorder

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Catechol-O-methyltransferase (COMT) plays a major role in the breakdown of catecholamines.¹ An amino acid polymorphism (val-108-met) determines high and low activity of the enzyme.^{2,3} A recent study in a small sample of patients with velo-cardio-facial syndrome who had bipolar affective disorder suggested that the Met (low activity) COMT allele might be associated with rapid-cycling in this population.⁴ We therefore tested the hypothesis that the Met allele might be associated with rapid cycling bipolar disorder in the wider population. We studied a sample of British Caucasian DSM-IV bipolar patients, of whom 55 met criteria for rapid cycling at some time during the illness and 110 met stringent criteria for a definite non-rapid cycling course. The COMT genotype was determined using a PCR assay. The low activity allele was more frequent in the group of rapid cyclers: 0.55 vs 0.42 (one-tailed $\chi^2 = 5.12$, d.f. = 1, $P = 0.012$), and bearers of low activity alleles showed a dose-dependent increased risk of lifetime occurrence of rapid cycling: χ^2 test of linear association = 4.84, d.f. = 1, $P = 0.014$. Our data support the hypothesis that variation in the COMT gene modifies the course of bipolar disorder.

Several lines of evidence suggest that catecholamines play an important role in the pathogenesis of bipolar disorder.^{5,6} Family, twin and adoption studies demonstrate the importance of a genetic contribution to the disorder but the mode of inheritance is complex and non-mendelian.^{7,8} Association studies in candidate genes are an important paradigm for genetic dissection of complex traits^{9,10} and genes involved in cat-

echolamine metabolism represent plausible candidates for study in bipolar disorder.

One such candidate is the gene encoding catechol-O-methyltransferase (COMT) which is an enzyme involved in one of the major degradation pathways for the catecholamine neurotransmitters (dopamine, adrenaline, noradrenaline), thereby inactivating them.¹ Approximately 20–25% of Caucasians express a low-activity variant of the enzyme. Homozygosity for the low-activity enzyme leads to a 3–4-fold reduction in enzymatic activity compared with homozygosity for the high activity enzyme, while heterozygotes have an intermediate activity.^{2,11–13} It has been shown that low activity is due to a G → A transition at codon 108 of the gene, leading to a valine → methionine substitution.^{2,3} It should be noted that COMT exists as two isoforms transcribed from alternative promoters, with the membrane-bound form having 50 more amino acid residues than the soluble form.¹⁴ Consequently the Val-108Met polymorphism has also been referred to as Val-158Met. The polymorphism, which results in the low-activity allele, creates a *Nla*III restriction site in the gene and can be detected using a PCR assay.

The presence of a functionally relevant polymorphism in the COMT gene makes it an interesting candidate for study in bipolar disorder. However, a large collaborative case-control association study on 412 bipolar I disorder patients and 368 controls demonstrated that variation at this functional polymorphism does not make an important contribution to susceptibility to bipolar disorder in the Western European population,¹⁵ and other groups similarly reported no association in 60 bipolar patients from the USA⁴ and 88 bipolar patients from Spain.¹⁶

A recent study on patients who had velo-cardio-facial syndrome (VCFS) and bipolar affective features found evidence that variation at the COMT gene may however modify the course of bipolar disorder, with the low-activity allele predisposing to rapid cycling.⁴ VCFS is a congenital disorder with an increased prevalence of major psychiatric disorders, particularly psychosis.^{17,18} Approximately 80–85% of patients with the disorder have a deletion involving chromosome 22q11. The COMT gene lies within the deleted region¹⁹ and most individuals with VCFS are therefore hemizygous (ie have only one copy) of the COMT gene. Lachman *et al*⁴ reported that all seven VCFS patients in their sample who had rapid-cycling bipolar affective disorder had only the low-activity COMT 108^{met} allele and speculated that this finding might generalise to bipolar patients without VCFS.

The hypothesis predicts that the allele which causes low COMT enzymatic activity will be more frequent among rapid cyclers when compared to individuals with a non-rapid cycling course of illness. We have tested this hypothesis by comparing COMT allele and genotype distributions in a UK Caucasian sample of DSM-IV bipolar patients.

The sample consisted of 165 patients: 55 satisfied criteria for lifetime occurrence of rapid cycling and 110 satisfied stringent criteria for lifetime non-rapid cyc-

Table 1 Characteristics of the bipolar patients divided according to course of illness

	<i>Non rapid cyclers</i> (<i>n</i> = 110)	<i>Rapid cyclers</i> (<i>n</i> = 55)
Gender (female/male)	58/52 (53% female)	34/21 (62% female)
DSM-IV diagnosis	104 BPI, 6 BPII	43 BPI, 10 BPII, 2 NOS
Positive family history	71 (66%)	40 (73%)
Age (s.d.)	46.2 years (13.4)	47.9 years (13.8)
Duration of illness (s.d.)	19.4 years (9.8)	17.1 years (11.0)

ling course (criteria described in the methods section). There were 92 female (56%) and 73 male patients. The sample consisted of 147 BP I, 16 BP II and two BP NOS patients. These last two patients had exhibited very rapid cycles of mood swings, of 48 h and 72 h, thus not qualifying for the 4 days duration criterion for BP II according to DSM-IV. Features of the RC and NRC groups are shown in Table 1. There was no significant difference between age, duration of illness, gender distribution or presence of a positive family history of treated psychiatric illness in first or second degree relatives between the two groups of patients although, as expected, rapid cyclers were more likely to be female (62% vs 53%) and much more likely to have a diagnosis of BP II: 10 of the 16 BP II and both BP NOS patients were rapid cyclers ($\chi^2 = 10.1$, d.f. = 1, $P = 0.0015$).

Table 2 shows the genotype and allele distributions in the RC and NRC groups in the present study and, to aid interpretation, also shows the distributions in British Caucasian controls from the same geographic areas, as reported previously by our teams.¹⁴

The frequency of the low activity allele was higher in RC (0.55) and lower in NRC (0.42) than in controls (0.49). The difference between RC and NRC was significant ($\chi^2 = 5.12$, d.f. = 1, $P = 0.012$). The genotype distributions were also significantly different in the predicted direction, with evidence of a dosage effect of the low activity allele (Mantel-Haenszel χ^2 test = 4.84, d.f. = 1, $P = 0.014$). A dosage effect is confirmed by consideration of odds ratios. Using the high activity homozygote as baseline, bearers of low activity alleles show a dose-dependent increased risk of lifetime

occurrence of RC: heterozygote: odds ratio (OR) = 1.83 (95% confidence intervals, CI = 0.81–4.14); homozygote: OR = 2.80 (CI = 1.4–7.07). The observation of a dose-dependent effect increases confidence that our finding reflects the biology of the disease process and is not a type I error. Separate analyses were performed for patients recruited in England and Wales and the effect was in the same direction and of a similar magnitude (data not presented), so sample stratification does not appear to have produced any bias.

We have used stringent criteria to select definite RC and NRC patients from a large sample of bipolar patients. Time-censored classification of individuals according to lifetime course of illness has obvious methodological problems (eg incomplete availability of data and limited length of observation period) and is inevitably an imprecise procedure. Traditionally, rapid cyclers have been compared with the residual bipolar patients in the samples, which have been classified as non-rapid cyclers.²⁰ Such a definition may include many marginal cases, cases for which data are inadequate and cases of recent onset who may not have had the opportunity to demonstrate the true life-time course of the disorder. Our approach which focuses on the most clear-cut members of each group is likely to minimise the noise inherent in such classification, and to provide increased power to detect an effect.

It is important to note that our group of patients as a whole did not differ from the control sample in genotype or allele frequencies. Thus our results are in agreement with previous findings^{4,15,16} which found no difference in allele or genotype frequencies between bipolar patients and controls. Thus, there is no evi-

Table 2 Genotype and allele frequencies (proportions in parentheses) of the COMT *NlaIII* polymorphism in rapid cycling patients, non-rapid cycling patients and controls

Population	<i>n</i>	Genotype			Allele	
		1 1	1 2	2 2	1	2
Non-rapid cyclers	110	38 (0.35)	51 (0.46)	21 (0.19)	127 (0.58)	93 (0.42)
Rapid cyclers	55	11 (0.20)	27 (0.49)	17 (0.31)	49 (0.45)	61 (0.55)
Total BP	165	49 (0.30)	78 (0.47)	38 (0.23)	176 (0.53)	154 (0.47)
Controls	161	41 (0.25)	83 (0.52)	37 (0.23)	165 (0.51)	157 (0.49)

Allele 1: COMT^{val} (high activity allele).

Allele 2: COMT^{met} (low activity allele).

dence that variation at the COMT gene influences *susceptibility* to bipolar disorder. This notion is compatible with previous research on rapid cycling, which showed that the morbid risk for affective disorder is not different in the relatives of rapid cycling and non-rapid cycling probands.^{20–22} These same studies have produced inconclusive results regarding the prevalence of rapid cycling in the relatives of bipolar patients with and without the rapid cycling form. In the presence of a relatively modest genetic effect, as suggested by our results, these studies had a low power to demonstrate a significant genetic effect.

It is clear from our results that variation in activity of COMT cannot be the only factor determining a rapid cycling course of illness. Other factors that have been suggested are female gender^{20–22} and the presence of thyroid disorder.²³ Exposure to tricyclic antidepressants has been consistently reported to induce rapid cycling. Studies and case reports prior to 1990 have been reviewed by Goodwin and Jamison⁵ and the same finding was reported in a later study.²⁴ These findings could provide a clue about the mechanism which might cause low-activity COMT to predispose to rapid cycling, as the tricyclics also increase the availability of catecholamines (noradrenaline and to some extent dopamine). It is plausible that gene-environment interactions occur and it will be important to undertake studies to explore this possibility. In the light of the current finding with COMT, other genes involved in monoamine metabolism and neurotransmission warrant investigation. These include the monoamine oxidases,²⁵ tyrosine hydroxylase, and the monoamine transporters, eg the functional polymorphism identified within the promoter of the serotonin transporter gene.²⁶

The patients in the study of Lachman *et al*⁴ are described as experiencing approximately 8–10 episodes per year. It is possible that in the hemizygous state the low activity allele exerts a particularly marked effect on rapid cycling due to the presence of only one copy of the COMT gene. It is also possible that low activity COMT may predispose to an even more pronounced rapid-cycling course, than that defined by DSM-IV criteria (four episodes per year) and our sample provides some support for this notion. Out of 16 patients who had eight or more regular alternating episodes for at least 2 years, eight were homozygotes for the low-activity allele, five were heterozygotes and three were homozygotes for the high activity allele.

In conclusion, our results support the hypothesis put forward by Lachman *et al*⁴ that low-activity COMT alleles can predispose to a rapid cycling course of illness in bipolar patients. Further studies using the same methods are required to confirm this association.

Methods

Subjects

Each member of a sample of UK bipolar patients recruited for molecular genetic studies who were white Caucasians of European origin was considered for

inclusion in the study. The total sample included 755 patients with bipolar affective disorder recruited from psychiatric outpatient and lithium clinics in England and Wales. All patients had given a written informed consent for participation in genetic linkage and association studies in psychiatric disorders.

The patients were classified into one of three categories on the basis of lifetime course of illness:

Definite lifetime occurrence of rapid cycling (RC)—A subject was classified as having lifetime occurrence of rapid cycling if he/she met DSM-IV criteria on at least one occasion (ie four or more episodes of mood disturbance in a 12-month interval, demarcated either by partial or full remission for at least 2 months, or a switch to an opposite polarity).

Definite lifetime non-rapid cycling course (NRC)—A subject was classified as having lifetime non-occurrence of rapid cycling if each of the following criteria was satisfied:

1. At least 7 years had elapsed since the onset of major affective disorder.
2. At least three distinct episodes of major affective disorder had occurred.
3. No more than two completed episodes of affective illness had occurred in any 12-month period.
4. More than 12 months had elapsed between the onset of any one episode and the end of the next episode of the same polarity.

Uncertain—All patients not meeting the criteria for either RC or NRC were classified as uncertain. This group contains the majority of subjects and includes subjects for whom insufficient information was available to make an unambiguous assignment to RC or NRC categories.

For all subjects included in the study best-estimate lifetime diagnoses according to DSM-IV were made by two independent clinicians on the basis of all available information which included a structured psychiatric interview: SCAN,²⁷ or SADS-L²⁸ and hospital case notes. It should be noted that we have previously demonstrated highly satisfactory agreement at the level of final diagnostic classification between data elicited by SADS-L and PSE/SCAN.^{29,30} The classification of patients into RC or NRC categories was agreed by two raters who were blind to knowledge of COMT genotype.

Genotyping

High molecular weight DNA was isolated from lymphocytes by routine procedures. The PCR assay used identifies an *Nla*III polymorphism, the restriction endonuclease site being present in the low-activity COMT (allele 2) and absent in the high-activity COMT (allele 1). Each individual was typed for high and low COMT activity by amplification of a 169-bp fragment with the primers: 5'-ACTGTGGCTACTCAGCTGTG-3' and 5'-CCTTTTCCAGGTCTGACAA-3'.

The following PRC conditions were used: 2 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM of each dNTP, 25 pM of each primer, 60 ng of genomic

DNA and 1 unit of Taq Polymerase (Amersham Life Sciences, UK). Temperature cycles were: 94°C for 5 min followed by 35 cycles of 57°C, 72°C and 94°C for 30 s each and a final elongation step at 72°C for 5 min. After digestion with the enzyme *Nla*III at 37°C for 2 h, the products were separated on 3% agarose gels and visualised by ethidium bromide staining and ultraviolet transillumination. All genotypes were assigned by three independent raters who were unaware of the subjects' clinical status.

Statistics

Statistical significance of differences between RC and NRC distributions for alleles and genotypes was assessed using χ^2 tests and, where appropriate, odds ratios and confidence intervals. Because the COMT genotypes have an ordered effect on enzyme activity, the Mantel-Haenszel χ^2 test for linear trend was used to assess a dosage effect of the low activity allele. Because the hypothesis tested specified a direction of effect, one-tailed *P*-values were used.

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