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FEATURE REVIEW

The catechol-*O*-methyl transferase (*COMT*) gene as a candidate for psychiatric phenotypes: evidence and lessons

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The enzyme catechol-*O*-methyl transferase (COMT), identified in the 1950s, is involved in catabolism of monoamines that are influenced by psychotropic medications, including neuroleptics and antidepressants. The *COMT* gene lies in a chromosomal region of interest for psychosis and bipolar spectrum disorder and a common polymorphism within the gene alters the activity of the enzyme. As a consequence, *COMT* has been one of the most studied genes for psychosis. On the basis of prior probabilities it would seem surprising if functional variation at *COMT* did not have some influence either on susceptibility to psychiatric phenotypes, modification of the course of illness or moderation of response to treatment. There is now robust evidence that variation at COMT influences frontal lobe function. However, despite considerable research effort, it has not proved straightforward to demonstrate and characterise a clear relationship between genetic variation at *COMT* and psychiatric phenotypes. It is of course, possible that *COMT* will turn out to be an unusually intractable case but it seems more likely that the experiences with this gene will provide a foretaste of the complexity of genotype–phenotype relationships that will be found for psychiatric traits. In this review, we consider the current state of evidence and the implications both for further studies of *COMT* and more generally for studies of other genes.
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Introduction

The enzyme catechol-*O*-methyl transferase (COMT), identified in the 1950s,[1] is involved in catabolism of monoamines that are influenced by psychotropic medications, including neuroleptics and antidepressants. The *COMT* gene lies in a chromosomal region of interest for psychosis and mood disorder and a common polymorphism within the gene alters the activity of the enzyme. As a consequence, *COMT* has been one of the most studied genes for psychosis. In this review we consider the current state of evidence and the implications both for further studies of *COMT* and more generally for studies of other genes.

Catechol-*O*-methyl transferase: enzyme and gene

COMT degrades catecholamines including dopamine. Two main COMT protein isoforms are known. In most assayed tissues, a soluble cytoplasmic (S-COMT) isoform predominates.[2] In brain, a longer membrane-bound form (MB-COMT) is the major species.[3] Although expressed widely, COMT appears to be a minor player in dopamine clearance compared with neuronal synaptic uptake by the dopamine transporter and subsequent monoamine oxidase (MAO) metabolism.[4] However, in the prefrontal cortex (PFC) where dopamine transporter expression is low,[5] the importance of COMT appears to be greater.[6,7]

The structure of the *COMT* gene, which lies on chromosome 22q11, is shown in Figure 1. A common G>A polymorphism is present that produces a valine-to-methionine (Val/Met) substitution at codons 108 and 158 of S-COMT and MB-COMT, respectively,[8] that results in a trimodal distribution of COMT activity in human populations.[8–10] The polymorphism is usually referred to as the Val/Met locus, but is also known by the reference sequence identification code rs4680 (previously rs165688). Terminology varies and can be confusing: the Valine (Val) allele is also referred to as the high activity (H) allele or the G allele. We will refer to it as the Val allele.

A number of putative regulatory elements have been discovered in the *COMT* gene, which may explain the differential expression of the long and short transcripts in different tissues.[3] These include numerous oestrogen response elements[11]

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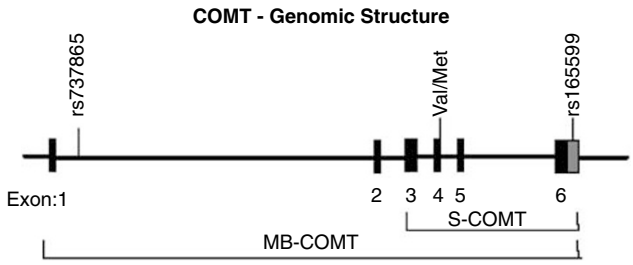

Figure 1 Schematic representation of *COMT* gene. The six exons of *COMT* are shown together with the and indication of the structure of the two transcripts, S-COMT and MB-COMT. The three polymorphisms that have been most widely studied are shown.

and oestradiol has been shown to downregulate COMT expression in cell culture.[12] A recent report suggests that MB-COMT exists in two forms which may be differentially affected by the Val/Met genotype.[13] Thus, it is to be expected that there will be a level of genetic complexity including possible gender-specific effects.

Polymorphism and haplotype frequencies at *COMT* have been shown to vary substantially across populations.[14,15] For example, the Val allele has been reported at frequencies varying between 0.99 and 0.48.[14] Moreover, in certain Asian populations, a second functional variant, Ala72Ser, (MB COMT nomenclature) has been reported[16] Hence, population origin of samples is a potentially important variable for interpreting genetic studies of *COMT*.

Positional studies of psychiatric phenotypes

Microdeletions

The 22q11 chromosomal region in which *COMT* is located is involved in microdeletions that are present in most individuals with velocardiofacial syndrome (VCFS) and related clinical syndromes which we will collectively refer to as *chromosome 22q11 deletion syndrome* (22q11DS). In addition to characteristic core features of dysmorphology, abnormalities of the palate and congenital heart disease, cognitive impairments are common, and range in severity from minimal to severe.[17] An increase in a broad spectrum of psychiatric disorders in children with 22q11DS has been reported including anxiety, mood disorders, obsessive-compulsive disorder (OCD), and attention deficit disorder.[17–21] In adults with 22q11DS, high rates of psychosis have been reported,[20,22–24] with the majority of cases satisfying diagnostic criteria for schizophrenia[25] and an estimated risk of around 25%.

Linkage studies

Linkage studies have provided evidence for one or more loci in the 22q11 region in which *COMT* is located that influence susceptibility to several psychiatric phenotypes.

Schizophrenia. The region is supported by both published meta-analyses of schizophrenia linkage.[26,27]

Bipolar disorder. The region is supported by one of the two published meta-analyses of bipolar disorder.[26]

Schizoaffective disorder. The only linkage study to use families selected on the basis of having at least one member with schizoaffective disorder, bipolar type provided genome-side suggestive evidence for linkage at 22q11.[28]

Other phenotypes. Many fewer linkage studies have been undertaken for other psychiatric phenotypes so there have not been the same opportunities for this chromosomal region to be implicated for these phenotypes. The region has not been implicated in genome scans of unipolar depression.[29] Lod scores close to 3 have been reported at the 22q11 locus close to *COMT* in a study of 70 panic disorder pedigrees.[30] This is of particular interest given that evidence has been reported to support a genetic contribution to the co-occurrence panic disorder in some bipolar disorder sufferers.[31]

In summary, on the basis of position, *COMT* must be considered as a strong candidate for involvement in psychiatric phenotypes, particularly psychosis and bipolar mood disorder.

Association studies of *COMT* and frontal lobe function

Several studies suggest that the *COMT* Val/Met locus influences performance on tests of frontal lobe function, with the Val allele and/or Val/Val genotype being associated with poorer performance. The Val allele was initially associated with poorer function as indexed by the Wisconsin Card Sorting Test and fMRI[32] in patients with schizophrenia and controls. Subsequently, a fairly strong body of evidence has been reported for association between the Val allele and poorer performance in controls,[33–35] patients with schizophrenia,[34,36,37] the sibs of schizophrenics (although not the schizophrenics themselves),[38] and a small sample of subjects with 22q11DS.[39] One study of individuals with schizophrenia demonstrated a biphasic effect, with the Val allele being associated with poorer performance in some tests, but better performance in others.[37] However, not all studies,[40,41] including the largest study comprising 543 Greek army conscripts,[42] have supported association between frontal cognitive measures and *COMT*. Recently, the low activity Met allele has been reported as a risk factor for cognitive decline in 22q11DS.[43]

Most work to date has focussed on the Val/Met polymorphism and other variants reported to be associated with altered mRNA expression[44,45] have not been widely studied. However, on the current evidence, the mechanism for the cognitive effects at *COMT* is unlikely to be simple. Weinberger and

colleagues have suggested that it is likely that the relationship between COMT activity and PFC function is more complex than simply 'Met[158] good, Val[158] bad'[46] and have argued in support of an inverted U-shaped relationship between dopamine levels and PFC function. The argument is that the precise effect of COMT activity on PFC function is likely to be dependent on where on the inverted-U curve the individual in question lies in any given environmental or genetic context. This is likely governed by multiple factors, including the nature of the measure being examined,[37] state factors, for example the relative amount of stress that the individual is under, which is known to affect PFC dopamine levels[47] and trait factors, such as the complex genetic background on which the *COMT* genotype is expressed, Under this model, 22q11DS can be considered as an example of the effect of genetic background, with the high activity Val allele being associated, in some studies, with better cognition[48] and less decline[43] because the higher Val activity rescues people with 22q11DS from the consequences of having only one gene copy.

In summary, there is strong evidence for an effect of *COMT* on cognitive function. Given that prefrontal cognitive function has been proposed as a trait marker for schizophrenia[32] this offers the potential for explanatory mechanisms of how variation in *COMT* may influence abnormal brain function in psychiatric phenotypes.

Association studies of *COMT* in psychiatric phenotypes

Perhaps not surprisingly most psychiatric association studies of *COMT* to date have involved only the functional Val/Met polymorphism and have focussed mainly on the phenotypes of schizophrenia and, to a lesser extent, bipolar disorder. However, some studies have been undertaken in a range of other phenotypes. Further, there is a recent trend towards study of other polymorphisms across this locus. We will consider the evidence for the better studied phenotypes below.

Schizophrenia – Val/Met

The *COMT* Val/Met variant has been one of the most studied candidate polymorphisms for schizophrenia. The vast majority of case–control studies have failed to find evidence for association. In a meta-analysis of studies predating August 2002,[49] only two[50,51] of 14 case–control studies yielded significant evidence for association (the Met allele in each case). However, when all studies were combined (total: 2205 cases; 2236 controls), the odds ratio (OR) for the Met allele was 1, indicative of no effect. Separate analysis of case–control samples by Asian or European origin also failed to provide evidence for association. Of five family studies included in the meta-analysis, two reported significant evidence for association, this time with the Val allele. In the meta-analysis,[49] the authors concluded that overall there was some support for association of schizophrenia with the Val allele in European samples.

An updated meta-analysis of case–control literature published prior to December 2003[52] including eight Asian studies (2125 patients, 2504 controls) and 11 European studies (1350 patients, 1573 controls) found no significant effect but a trend for over-representation of the Val allele in cases (OR = 1.09, confidence interval (CI) 0.94–1.26). A further meta-analysis using the December 2003 data but using a regression approach found significant evidence for association of the Val allele with schizophrenia if all studies were included but loss of significance if studies were excluded where the control sample showed departure from Hardy–Weinberg equilibrium (suggesting the possibility of genotyping error or population stratification).[53] Studies published subsequently include our own study of two large association samples from the UK (709 cases, 710 controls) and Bulgaria (488 parent–proband trios),[54] which found no support, and data from a Korean study of around 300 cases and 300 controls which was similarly negative.[16] A recent study of Turkish cases ($n = 297$) and controls ($n = 341$) suggested the Met/Met genotype as a risk factor for schizophrenia[55] but caution is required because controls departed very substantially from Hardy–Weinberg expectations.

In summary, despite the investigation of many thousands of schizophrenia cases and controls no consistent, significant evidence for association at the Val/Met locus has emerged. Meta-analyses have provided (nonsignificant) estimates of an over-representation of the Val allele with an effect size of approximately 1.1.

Other polymorphisms

A number of groups have sought evidence for susceptibility variants elsewhere in the gene. Some of the strongest positive evidence was reported in a large study of Ashkenazi Jews[56] comprising around 700 cases and approximately 3000 controls. Modest evidence was found for association between the Val allele and schizophrenia ($P = 0.024$) but two other polymorphisms, rs737865 in intron 1 and rs165599 within the 3′UTR of some COMT mRNA species[44] (see Figure 1), were more strongly associated. The haplotype carrying the G allele at all three loci (which at Val/Met encodes Val) was also strongly associated ($P = 9.5 \times 10^{-8}$). Interestingly, the three other haplotypes carrying the Val allele were under-represented in cases. Gender effects were also reported although these are difficult to interpret because much of the effect was driven by differences in the controls, not the cases.

Several studies have subsequently examined a range of markers including those required to define the 'Shifman haplotype' (none were included in the meta-analyses discussed above). One based on 267 Irish multiplex families[57] revealed modest evidence for excess transmission of the Val allele using a broad case definition including schizophrenia and mood-

psychosis spectrum phenotypes ($P=0.01$). Haplotype analysis provided marginally stronger evidence than Val/Met alone. Only one of four relatively common haplotypes carrying the valine allele was significantly overtransmitted (A-G-A) to schizophrenics, while one, the G-G-G risk haplotype of Shifman, was significantly *under*-transmitted indicating a protective effect.

Sanders *et al.*[58] examined eight markers spanning COMT and extending into the neighbouring gene, *armadillo repeat gene deleted in velocardiofacial syndrome* (*ARVCF*), in 136 families of mainly European American origin. Several haplotypes including markers reaching into *ARVCF* yielded significant evidence for association (best nominal global $P\sim0.002$). The individual specific haplotype displaying the excess transmission was almost fully characterised by G-A at Val/Met-rs165599 and is, therefore, consistent with the associated A-G-A rs737865-Val/Met-rs165599 haplotype of Chen *et al.*[57]

In a sample of 50 white Australian affected sib-pairs evidence (permuted global $P\sim0.002$) was obtained using haplotypes of the three Shifman markers.[59] Much of the association appears to have been driven by *under*-transmission of the A-G-A (Val containing) haplotype (transmitted = 1, nontransmitted = 18). A number of gender-specific findings were observed but caution is required given the small sample sizes, and issues of multiple testing (although methods for accommodating this as well as controlling for multiple testing were still positive according to our communications with the authors).

Finally, in the largest single study of *COMT* including almost 1200 cases representing a case ($N=709$) control ($N=710$) sample from the UK and a family-based association sample of complete trios ($N=488$) from Bulgaria, our own group found no evidence for association in either sample to the Val/Met locus or to any of the Shifman markers or haplotypes.[54] Analysis by gender also failed to identify any evidence for association to markers or haplotypes.

In summary, our own negative study nothwithstanding, the recent data based upon additional polymorphisms and haplotypes provide some encouragement that variation at *COMT* influences the schizophrenia phenotype. However, robust replication is required preferably using genewide tests of significance.[60] Further, the data suggest a mechanism that involves substantial complexity over and above any effect of the Val/Met polymorphism itself and could involve variation in neighbouring *ARVCF*.

Studies of *COMT* in bipolar disorder

Val/Met

Although less studied than in schizophrenia, the Val/Met polymorphism has received substantial investigation in bipolar disorder and, like schizophrenia, the findings are inconclusive or negative. Meta-analysis of the seven case–control studies in the literature in 2001 (910 bipolar cases, 1069 controls) provided borderline significant evidence for association of the Met allele with susceptibility to bipolar disorder (OR = 1.18, CI 1.02–1.35).[61] However, an updated meta-analysis undertaken as part of the current review (2169 bipolar cases, 7804 controls; Figure 2) provides no evidence to support association, although a nonsignificant trend towards association with the Met allele remains (OR = 1.08, CI 0.94–1.24; $P=0.30$). No evidence for association at *COMT* Val/Met has emerged from family-based association studies[62–64] but only a few hundred families have been reported so this observation must be interpreted within the context of very limited power.

Analyses of bipolar samples have been undertaken with the Val/Met polymorphism to seek evidence for association with clinical subphenotypes. Positive

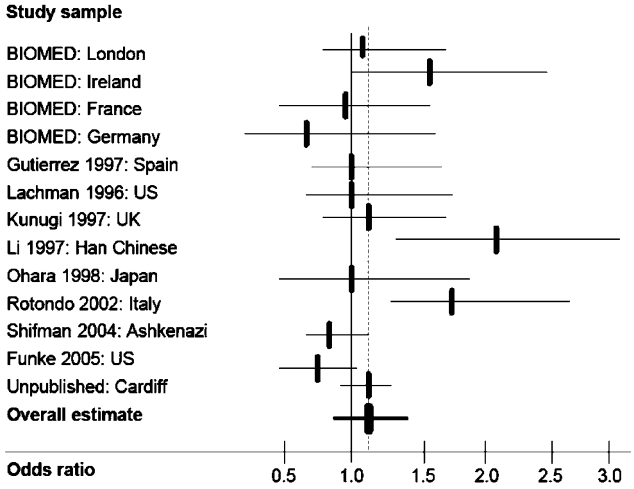

Figure 2 Forest plot for meta-analysis of studies of *COMT* Val/Met polymorphism in bipolar disorder. Meta-analysis of studies of bipolar disorder reported in the English literature available by PubMed search on 1 December 2005, together with unpublished data from our own group. Only non-overlapping data sets with sample size exceeding 20 cases were included. A random effects model was used in analyses undertaken with the software EasyMA 98b.[117] Overall estimated effect sizes: Odds ratio in favour of overrepresentation of Met allele, OR = 1.08 (0.94–1.24) ($P=0.30$). There was no evidence for heterogeneity across studies ($P=0.26$) and funnel plot showed no evidence for publication bias (data not shown). The samples, with ethnic origin, included in the meta-analysis were as follows: BIOMED London:[118] 101 cases, 86 controls; BIOMED Ireland:[118] 87 cases, 87 controls; BIOMED France:[118] 95 cases, 91 controls; BIOMED Germany:[118] 28 cases, 27 controls; Gutierrez *et al.*, Spain:[119] 88 cases, 113 controls; Lachman *et al.*, US:[120] 63 cases, 87 controls; Kunugi *et al.*, UK:[121] 107 cases, 127 controls; Li *et al.*, Han China:[122] 93 cases, 98 controls; Ohara *et al.*, Japan:[74] 40 cases, 135 controls; Rotondo *et al.*, Italy:[81] 111 cases, 127 controls; Shifman *et al.*, Ashkenazi:[68] 214 cases, 4018 controls, Funke *et al.*, US:[101] 82 cases, 467 controls; Cardiff unpublished: 1030 cases, 2347 controls. The forest plot shows the estimated effect size in thick vertical line and the 95% confidence interval as a thin horizontal line.

reports include significant over-representation of the Met allele in rapid cycling.[65–67] Although interesting, these reports must still be considered preliminary because the findings have not yet been subjected to widespread attempts at replication.

Other polymorphisms

As for schizophrenia, some groups have sought evidence for susceptibility variants elsewhere in the gene. Little has been published and no compelling positive evidence has yet emerged but the modestly positive report by Shifman *et al*.[68] is of interest and suggests the possibility that the same risk haplotype that confers risk of schizophrenia in the Ashkenazi population may also confer risk of bipolar disorder.

Studies of *COMT* in other phenotypes

Catechol-*O*-methyl transferase has received limited study in a range of other psychiatric phenotypes. Typically, studies are few in number, samples are small and only the Val/Met polymorphism has been considered. Many reports involved substantial multiple testing with examination of a variety of phenotypic definitions or traits, as well analyses in the total sample and the sample divided according to gender. These issues make it difficult to draw any firm conclusions. Within the scope of this review, we can only illustrate the current state of the literature by giving examples for some of the better studied phenotypes.

Obsessive-compulsive disorder

The group of Karayiorgou aroused great interest when they reported association of susceptibility to OCD with the *COMT* low activity Met allele in 73 cases and 148 controls[69] with maximum effect in males. However, a meta-analysis of seven studies published up to 2003 (total: 144 cases; 337 controls; 269 trios) concluded that there was insufficient evidence to support association, with or without a gender effect.[70] Two subsequent studies (total: 158 cases; 373 controls) also failed to provide significant evidence for association[71,72] although the second of these found a nonsignificant trend for excess Met allele in male OCD cases and the same effect in a sample of 113 schizophrenia patients with comorbid OCD.[72]

Unipolar depression

No consistent findings have emerged from the few studies of unipolar samples (most of 100 or less cases) that have been published. A recent study found evidence for association of the Val allele with early onset depression in a sample of 120 patients with onset below 25 years of age but not in the larger set of 378 cases unselected for age at onset.[73] The other positive finding reported was association of the Met allele with unipolar depression in a small Japanese sample (75 cases, 135 controls).[74]

Attention deficit hyperactivity disorder

No evidence for association has emerged from studies of Val/Met undertaken to date,[75–78] which include 458 trios of European origin and 281 families of Asian origin.

Panic disorder

Borderline significant excess of the Met/Met genotype was reported for the phenotypes of panic disorder, trait anxiety and treatment response in a sample of 178 panic disorder cases and 182 controls.[79] In contrast, excess Val allele was reported in 115 panic cases compared with matched controls,[80] an effect present in females but not males. Interestingly, comorbid panic disorder has been suggested as a marker of a genetically homogeneous subphenotype of bipolar disorder[31] so a possible effect of the *COMT* locus on both panic disorder and bipolar disorder could mediate this relationship.[81] However, inconsistent with this attractive possibility, in a study of 111 bipolar disorder cases Rotondo *et al*.[81] found evidence for association of the Met allele with bipolar disorder only in cases *without* comorbid panic disorder.

Other phenotypes

Other phenotypes studied include phobic anxiety (sample: 1234 females; Val allele associated),[82] persistent anxiety (sample: 962 individuals; Met allele and Met/Met genotype associated in females),[83] nicotine dependence (positive and negative studies reported)[84] and anorexia nervosa (association reported in 66 restricting anorexics but not 19 with bingeing/purging subtype).[85]

To summarise, in general reports lack consistency and no clear pattern has emerged. For each phenotype domain (such as anxiety spectrum disorders) a formal meta-analysis is required that includes all available data (published and unpublished) and takes account of the relationships between the phenotypes examined and the level of multiple testing. Almost certainly, substantially larger and more systematic studies will be required before the true state will emerge.

Gene–environment interactions

It is expected, essentially by definition, that the pathogenesis of genetically complex disorders, including psychiatric phenotypes, will involve interaction between multiple genes and environmental factors. [86] If possible it is highly desirable to include interacting environmental variables within analyses because, under ideal circumstances, this optimises the power to identify both the genetic and environmental factors. However, in general this proves difficult because of lack of knowledge and data on environmental variables of relevance. Further, inclusion of irrelevant variables actually reduces power as a result of the requirement for correction for multiple testing and the introduction of random variation.[87]

Within this context, two recent reports support the possibility of gene–environment interactions involving the *COMT* Val/Met polymorphism. First, in a longitudinal study of the Dunedin birth cohort followed to adulthood carriers of the *COMT* Val allele were most likely to exhibit psychotic symptoms and to develop schizophreniform disorder if they used cannabis, whereas cannabis use had no such adverse influence on individuals with two copies of the Met allele.[88] This observation is consistent with *COMT* modulating the psychotogenic effects of cannabis exposure. Second, in a family-based association study of 240 ADHD sufferers, bearers of the Val/Val genotype were substantially more susceptible to the adverse effects of prenatal risk (indexed by low birth weight) in influencing risk of early-onset antisocial behaviour.[89] This observation is consistent with modulating effects of *COMT* and prenatal factors on the known relationships between prefrontal cortical dysfunction and antisocial behaviour.

These findings require replication but suggest the possibility that gene–environment interactions may contribute to the complexity genotype–phenotype relationships at *COMT*.

Interpretation of data for psychiatric phenotypes

It will be readily apparent to the reader that few unambiguous or simple findings have emerged from studies to date of variation at *COMT* for psychiatric phenotypes (Table 1). It is not appropriate to consider most phenotypes in detail because of the limited work undertaken. Indeed, for these phenotypes the requirement is for more studies, larger samples and systematic coverage of the gene (Table 1). However, it is useful to consider how to interpret the current body of work available for psychosis and bipolar mood disorder.

Table 1 Key conclusions from current data on *COMT* and psychiatric phenotypes

- A strong body of data supports an effect of the *COMT* Val/ Met locus on frontal lobe function (Val associated with poorer function)
- Both positional and functional evidence makes the *COMT* gene a strong *a priori* candidate for involvement in psychosis and other psychiatric phenotypes
- There has been substantial study of schizophrenia and to a lesser extent bipolar disorder, at least for the Val/ Met polymorphism
  - A single, simple main effect of Val/ Met can be excluded for schizophrenia and bipolar disorder
  - Positive findings from studies of multiple polymorphisms are promising and appear to be more common than expected by chance alone
  - Despite more extensive study, the genetic evidence for the involvement of *COMT* in psychosis is less compelling than for dysbindin, neuregulin 1, DISC1 or DAOA (G72)
  - The optimal clinical phenotype definition for studies of *COMT* is not yet known
- Phenotypes other than schizophrenia and bipolar disorder have yet to be studied in large samples
- For all phenotypes, there is a requirement for more studies, larger samples and systematic analysis of variation across the gene

Schizophrenia

This is by far the best studied phenotype. The default hypothesis to consider must be the null hypothesis. The meta-analyses,[49,52,53] and two of the three largest single studies[52,54] do not allow this to be rejected. However, in striking contrast, several recent studies employing multiple markers do suggest association that, at least on the face of it, appears to be at a level beyond that expected by chance alone. These data therefore suggest it is still fairly plausible, although not proven, that a susceptibility locus exists at or around *COMT*. However, there are several caveats. First, haplotype analysis is highly sensitive to genotyping errors and can result in false positive and negative associations. Second, haplotype analyses provide opportunities for extensive multiple testing and, in the absence of a clear and systematic analytic strategy specified *a priori* it can be difficult to interpret the true level of statistical significance. Third, in such indirect association analyses the aim is to detect polymorphisms or haplotypes in linkage disequilibrium with pathogenically relevant variants (rather than the variants themselves). Under this approach it is to be expected that the pattern of findings will vary between studies because of differences in population structure. However, this requires a genewide approach to analysis in order to minimise both type I and type II errors.

Most work on *COMT* has been predicated on the hypothesis that the Val/Met polymorphism is a direct risk factor for schizophrenia. The classic hypothesis that schizophrenia results from enhanced dopaminergic neurotransmission[90,91] predicts that the Met allele will be directly associated, a prediction also suggested by the observation that deletion of *COMT* is associated with the high rate of psychosis in 22q11DS. In contrast, the hypothesis that excess dopamine function in the mesolimbic system is secondary to low dopamine function in the prefrontal cortex (e.g.[92,93]) predicts association to the Val allele as do reported associations between the Val allele and poor prefrontal function and between poor prefrontal function and schizophrenia.[32]

On the face of it, these competing hypotheses should be unambiguously resolved by analysis of Val/Met which should be straightforward because direct association analysis does not depend on linkage disequilibrium (LD) and therefore the results should be robust to the variable LD structure at *COMT*.[15] The findings should also be relatively robust to ethnic variation because in populations *showing any effect*, the same allele should be associated, although the effect size may vary.

However, the overall evidence from published studies is not compatible with either simple hypothesis. In addition to the overall balance of the single locus data, it is notable that in all of the studies in which an effect at Val/Met has been detected, where additional markers have been typed, it has been possible to subdivide haplotypes carrying the risk allele into risk, neutral, and even in some cases, protective haplotypes. As four of these have been family-based studies, this cannot be attributed to stratification. Moreover, in the only study[59] to present a formal analysis, the model including additional markers was significantly more significant than that restricted to the Val/Met locus alone, whereas the conditional analysis suggested two independent effects. These findings suggest that if function at the Val/Met locus is at all relevant to schizophrenia, the relative functional properties of the Val and Met alleles can be modified, even reversed, by at least one other relatively common *cis*-acting variant with an influence perhaps on *COMT* expression or splicing. This suggestion is broadly compatible with the demonstration of *cis*-acting loci that modify the expression of *COMT* mRNA independent of the Val/Met locus.[44,45] However, there are also data that argue strongly against the existence of more than one even moderately *common* functionally relevant polymorphism in *COMT*. Thus, while Chen *et al*.[10] demonstrated that brain COMT enzyme activity is associated with the Val/Met locus, this did not correlate with the markers that we and others have associated with mRNA expression[44,45] or with schizophrenia. This finding is based on subjects of European and African ethnic origins[10] although in certain Asian populations, a second functional Ala72Set variant has been reported.[16] As enzyme activity can be reasonably assumed to be functionally more important than mRNA abundance, the findings of Chen *et al*.[10] provides a strong refutation to our hypothesis[44] that the associations with the Shifman haplotypes are likely to be attributable to major or minor alterations in *COMT* expression. Unfortunately, it is difficult to obtain an exact estimate of the proportion of the variance in brain COMT activity that can be attributed to the Val/Met locus from that study given confounders like post-mortem variance, age, and measurement error. However, earlier studies with respect to peripheral COMT activity suggest that all, or almost all, COMT activity can be attributed to Val/Met.[8,94]

If it is correct that Val/Met is responsible for all almost all variance in COMT function, then if they indicate true association, the haplotype data must point to the involvement of another gene in LD with *COMT*. A strong candidate here is *ARVCF*[58,95] which shares common exonic sequence with COMT but on opposite strands. Moreover, one of the Shifman markers (rs165599) is located in the common exonic sequence and appears to influence *ARVCF* expression (Bray *et al*.[44] and unpublished data). That evidence for association to schizophrenia at a locus as *a priori* highly plausible as *COMT* might be attributable to association to an adjacent gene might seem an unlikely turn of events. It is, however, more parsimonious than a model which specifies that the Val/Met contributes to risk, but a second polymorphism in an adjacent gene dictates whether the Val or the Met allele is associated and does so without influencing COMT activity, as is implied if the Val/Met is the only locus with an important effect on COMT activity. However, if as may be the case for some tests of executive functioning, the risk of schizophrenia shows an inverted U-shaped dose–response with dopamine activity, one can envisage circumstances where such a complex model might apply and therefore this latter model should not be entirely discounted.

Taken as a whole, the current evidence is suggestive of some involvement of variation at or near *COMT* in predisposition to schizophrenia but, despite greater study, the level of evidence is less convincing than that available for several of the other genes of current interest, including *dysbindin, neuregulin 1, DISC1* and *DAOA(G72)*.[96,97]

Bipolar disorder

Substantially less work has been reported for bipolar disorder than schizophrenia. However, as for schizophrenia, it is clear that there is no simple single Val/Met effect. The nonsignificant trend from meta-analysis is for over-representation of the Met allele in bipolar cases compared with controls. This trend is in the opposite direction to that for schizophrenia and of similar magnitude (OR$\sim$1.1). Clearly this is consistent with the null hypothesis of no effect. It is, however, worth considering the possibility that Val/Met operates as a modifying locus for psychosis rather than a susceptibility locus, the Met allele increasing expression of positive and affective symptoms and the Val allele increasing expression of negative symptoms and cognitive dysfunction. This would be consistent with the mounting evidence linking the Val/Met variant with variation in cognitive and behavioural function (see above), but also some emerging but inconclusive findings suggesting it may influence response to medication (e.g.[98,99]).

Insufficient work has been reported in bipolar disorder for variants other than Val/Met to allow useful comment. Large systematic studies are needed.

Schizoaffective disorder

It has been conventional in studies of psychosis to recruit and investigate samples meeting criteria for either schizophrenia or bipolar disorder. Cases at the interface between these prototypical categories are common in clinical practice but have not been a focus of study. Few samples of Diagnostic and Statistical Manual of Mental Disorders (4th Edition) DSMIV Schizoaffective disorder are available although existing samples of DSMIV Bipolar disorder and schizophrenia include a diverse range of clinical picture and a variable proportion of cases that have a substantial

mix of the features of bipolar and schizophrenia prototypes. Several pieces of evidence suggest that the *COMT* locus, rather than conferring some general susceptibility to schizophrenia and bipolar disorder, may specifically influence susceptibility to an intermediate form of mood-psychosis phenotype:

(1) Genomewide suggestive linkage at 22q11 was found in a linkage study of families selected through a member with DSMIV schizoaffective disorder, bipolar type[28] (but not in the larger samples of schizophrenia and bipolar disorder families from which these were drawn).
(2) Linkage at 22q11 has been demonstrated within a subset of bipolar pedigrees in which individuals also experienced psychotic features.[100]
(3) The schizophrenia association finding of Chen *et al*.[57] at *COMT* was maximal when a broad spectrum of phenotype was used (including also mood-psychosis spectrum cases).
(4) Funke *et al*.[101] found evidence for association at *COMT* with individuals meeting criteria for schizoaffective disorder as well as those meeting criteria for schizophrenia and mood disorders.
(5) In our own study of over 1575 mood-psychosis cases and 2309 controls we found no evidence for association at *COMT* for the categories of bipolar disorder or schizophrenia. However, in the set of 308 cases who had experienced manic episodes and prominent psychotic features in at least half of all episodes of illness, we identified significant evidence for association (unpublished, presented at World Congress of Psychiatric Genetics, Boston[102]).

These observations raise the possibility that clinical heterogeneity between samples may be an important, perhaps major, cause for the observed inconsistencies in findings between studies.

General issues: lessons of relevance to psychiatric genetics

Functional and positional evidence make *COMT* a strong candidate for involvement in multiple psychiatric phenotypes (particularly, but not restricted to, schizophrenia and bipolar disorder). There is a common functional variant that exerts substantial influence over enzyme activity. On the grounds of prior probability, it could be considered an almost ideal candidate for being able to demonstrate or refute genetic association.[103] And yet, although it has been one of the most studied genes to date, no clear, unambiguous pattern of results has emerged. The experiences with *COMT* illustrate some general lessons that we can take forward for study of other genes (Table 2):

Table 2 Some key issues of relevance for gene studies of psychiatric phenotypes

- Adequate sample sizes are needed that are powered to detect realistic effect sizes (*N*'s closer to 1000 than 100)
- Any individual study cannot be expected to provide definitive answers and data should always be presented to facilitate meta-analysis
- Rather than study of single polymorphisms, systematic study of a gene/ locus should be the usual unit of analysis with appropriate genewide corrections made for statistical significance
- Detailed information on the distribution of clinical phenotypes in samples should be provided to supplement diagnostic categories
- Researchers should be open to considering nontraditional phenotype definitions and embrace an iterative approach to defining genotype–phenotype relationships
- Biological plausibility, whilst important, should not be allowed to compensate for poor genetics; conversely lack of biological plausibility should not detract from the importance of robust genetic findings
- Both phenotypic and genetic complexity should be anticipated, including the possibility of important gene–environment and/ or gene–gene interactions
- Close attention should be paid to estimating genotyping error rate and consideration of its possible effect on type I and II errors
- The possibility should be considered that a gene exerts its major role through phenotype modification rather than phenotype susceptibility

(1) Adequate sample sizes
Samples need to be adequately powered for plausible effect sizes.[104] Sample numbers need to be closer to 1000 than to 100. It is known that reliable replication of an effect typically takes samples several times larger than that in which the original observation was made.[105] The true state of affairs for each phenotype is unlikely to be clear until several thousand samples have been studied. An example is that of the Pro12Ala polymorphism at the gene encoding peroxisome proliferative activated receptor, gamma (*PPARG*) in type 2 diabetes where the first attempts at replication failed but when several thousand cases had been studied a robust (but modest) effect was established.[106]

(2) Importance of meta-analysis
Given the large samples required, meta-analysis is a crucial technique for summarising available data. Access to all relevant findings including negative data is required. All publications should, therefore, include the basic allele and genotype counts for each of the studied groups. Unfortunately, some reports have failed to include these because they use, for example, regression methods (e.g.[73]) and this diminishes their utility to the scientific community.

(3) Gene-based approaches
The study of the *COMT* Val/Met polymorphism in isolation probably continues to be justified because of its clear and major functional significance. However, it is clear that if *COMT* variation is important in psychiatric phenotypes, variants elsewhere within or

even outwith the *COMT* gene play an important, and possibly larger, role. Thus, the research focus should shift to study of the gene or locus as the unit, rather than a single polymorphism. Resources and methods are available that allow systematic coverage of a high proportion of the genetic variation at a locus and this gene-based approach offers many advantages including protection against genetic heterogeneity (i.e. different variants having maximal effects in different populations).[60]

(4) Closer attention to the clinical phenotype
Growing evidence suggests that genetic susceptibility will not respect current operational diagnostic boundaries.[96,97,107,108] Most samples are defined according to DSMIV diagnostic categories. There is an implicit assumption that, for example, one schizophrenia sample should be pretty similar to another. However, the schizophrenia phenotype is so broad that it is possible for one sample to be composed of chronically disabled individuals with cognitive impairment, marked negative features and minimal affective or positive psychotic symptoms whereas another sample could include relatively well functioning individuals with an episodic course and marked affective and positive psychotic symptoms. At the very least, it is important that substantial additional clinical detail is provided about samples (perhaps as on-line supplements) in order to allow meaningful comparisons between studies.

(5) An iterative approach
It is entirely possible that a relatively large effect size within a subset of the data is hidden amongst an overly broad traditional diagnostic group. It is, therefore, important that when a signal is identified researchers explore their data to refine the associated phenotype (clearly in the first experiment the exploration must be treated with caution but it can be tested explicitly in attempts at replication). This type of iterative approach (Figure 3) is important for optimising power to replicate genetic findings and has a wider importance in terms of moving to a more valid psychiatric nosology.[97]

(6) Biology is complementary, not superior, to genetics
High impact journals typically require 'biological support' for genetic association findings, presumably under the presumption that this provides enhanced credibility. However, the findings with *COMT* demonstrate that compelling biological plausibility does not ensure that genetic findings can be easily replicated or validated. Given the limited understanding of the pathophysiology of psychiatric disorders and the complexity of brain biology it is important that an appeal to biological plausibility is not allowed to exaggerate the significance of weak genetic data. Further, it is important not to lose sight of the fact that a major attraction of genetic studies of psychiatric phenotypes is the potential to discover entirely novel mechanisms of pathogenesis. To this end, it should be hoped that there *will* be entirely robust genetic findings that cannot be readily explained at a functional level. In terms of eventual impact on patient care, such observations are likely to prove to be the most important findings that will emerge from psychiatric genetics.

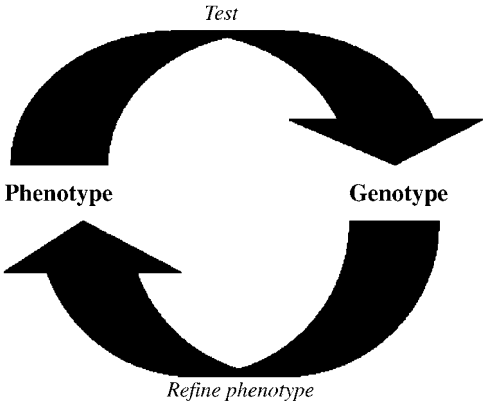

Figure 3 An iterative approach to psychiatric genetics. Schematic diagram illustrating an iterative approach to identifying and characterising susceptibility genes for psychiatric phenotypes. Some starting clinical phenotype must be chosen (perhaps a DSMIV category – although more informed definitions may be available). Positive genetic association signals can be used to define the optimal phenotype, which can be used in studies of independent samples. The procedure can be repeated to refine the genotype–phenotype relationship and identify biologically valid phenotypic sets.

(7) Closer attention to data quality
The most recent meta-analysis of the *COMT* Val/Met polymorphism in schizophrenia[53] demonstrates that conclusions can be influenced substantially by consideration of the quality of the data included. Genotyping error can be a cause of both type I and type II errors[109–115] in case control and family-based association studies. Haplotype analyses are particularly susceptible to genotyping errors[116] because errors can create spurious evidence for rare haplotypes. Any systematic difference in error rate between cases and controls (as could occur as a result of differences in DNA quality or purity) has the potential to generate spurious case–control differences on haplotype analysis. It is obviously important that this is recognised and that error rates are minimised. However, even in the best laboratories error rates are not negligible. Estimated error rates should always be reported and their possible impact on findings considered. Particular caution is needed for findings where significance comes entirely from very rare haplotypes.

(8) The detection of modifier loci
The inconsistent data relating genetic variation in *COMT* to specific phenotypes might reflect the fact that it is a modifier rather than a risk locus. This gains plausibility from the relatively more convincing data

relating *COMT* to cognitive function. Few if any association studies are undertaken with samples of incident cases and differing ascertainment procedures for prevalent cases is likely to lead to differences in factors such as severity, chronicity, and treatment that may be influenced by genes that do not increase risk of the disorder. The identification of modifier loci could have important implications for understanding pathogenesis and the design of novel therapies. However, it is perhaps important that researchers are able to distinguish modifier from risk effects relatively early on to avoid the plethora of inconsistent association studies with prevalent cases.

Conclusions

As a consequence of both its chromosomal location in a region of interest for psychosis and mood disorders and its function as an enzyme involved in catabolism of monoamines, *COMT* has been one of the most studied genes for psychosis. On the basis of prior probabilities it would seem surprising if variation at *COMT* did not have some influence either on susceptibility to psychiatric phenotypes, modification of the course of illness or moderation of response to treatment. There is now robust evidence that variation at *COMT* influences frontal lobe function. However, despite considerable research effort, it has not proved straightforward to demonstrate and characterise a clear relationship between genetic variation at *COMT* and psychiatric phenotypes. It is of course, possible that *COMT* will turn out to be a particularly challenging case but it seems more likely that the experiences with this gene will provide a foretaste of the complexity of genotype–phenotype relationships that will be found for psychiatric traits.

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