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COMT Contributes to Genetic Susceptibility Shared Among Anxiety Spectrum Phenotypes

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Abstract

Background—Catechol-O-methyl transferase (COMT) has been investigated for its possible role in a wide range of psychiatric phenotypes. In particular, several studies support association of this gene with panic disorder and other anxiety-related traits.

Methods—We examined the *COMT* gene for association with genetic risk across a range of anxiety spectrum phenotypes. We used multivariate structural equation modeling to select twin pairs scoring at the extremes of a latent genetic risk factor shared by neuroticism, several anxiety disorders, and major depression from a large population-based twin sample. Using one member from each of these pairs, the resulting sample of 589 cases and 539 controls were entered into a two-stage association study in which genetic markers were screened in stage 1, the positive results of which were tested for replication in stage 2.

Results—The functional val158met polymorphism (rs4680) plus nine other SNP markers selected to capture the major allelic variation across the *COMT* locus were analyzed for differences between cases and controls. While the val (G) allele of rs4680 showed marginally significant association in our combined stage 1 plus stage 2 sample, a high-risk haplotype of this allele with the A allele of rs165599 was significantly over-represented in cases ($p=1.97e-5$, OR=1.95). This haplotype also predicted individual differences in neuroticism and risk for several anxiety disorders and major depression. Consistent with prior studies, our findings are female specific.

Conclusions—Variations in the *COMT* gene contribute to genetic risk shared across a range of anxiety-related phenotypes.

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Keywords

catechol-O-methyl transferase; depression; anxiety; personality; association study; genetics

Introduction

Catechol-O-methyltransferase (COMT), the enzyme involved in the inactivation of catecholamines, has been investigated for its potential role in a broad range of psychiatric phenotypes (1). This gene possesses a common G>A functional polymorphism identified at codon 158 that produces an amino acid change from valine to methionine (2), commonly known as val158met (dbSNP rs4680). Accompanying this is a significant difference in the level of COMT enzymatic activity, with the val allele (G) possessing the higher activity.

The *COMT* gene has been implicated in pathological anxiety states from animal and clinical studies in a sexually dimorphic manner. Female mice in which *COMT* was disrupted exhibited changes in anxiety-related behaviors, while their male counterparts were affected in measures of aggression (3). Table 1 summarizes available studies of the *COMT* val158met polymorphism in relation to human anxiety-related symptoms or personality traits. The overall findings suggest a marginal and somewhat inconsistent association between the met allele or met-met genotype and higher levels of anxiety-related measures in females. Two exceptions are the McGrath et al. study (4) and the Kim et al. study (5), both of which reported higher anxiety-related scores in association with the val allele in females. In the only study to examine, for anxiety-related measures, other sources of variation in the *COMT* locus than val158met, Stein and colleagues studied three SNPs (including rs4680) that define a haplotype associated with variation in brain expression of COMT (6) in relation to NEO personality traits neuroticism and extroversion (7). They found lower extroversion and higher neuroticism in females with the met allele, with this relationship becoming more highly significant in haplotypic analyses with combinations of the three SNPs. Prior studies in schizophrenia have also reported multi-marker haplotypes that showed greater association with the phenotype than the val158met polymorphism alone (1;8).

The *COMT* val158met polymorphism has been tested for association with several anxiety disorders, with panic disorder demonstrating the most consistent findings. The group at Columbia University analyzed *COMT* for linkage and association with panic disorder in their family-based Caucasian sample of 70 panic disorder pedigrees and 83 parent-offspring triads (9). They found significant linkage for several polymorphisms, including val158met, as well as significant association for several haplotypes made up of combinations of these polymorphisms, finding the high-activity val allele associated with panic disorder. A recent review and meta-analysis of six case-control studies of val158met in relation to panic disorder reported an overall significant association of the val allele with panic disorder in Caucasian samples but a trend towards association of the met allele in Asian samples; their sub-analysis by gender suggests that the association is limited to females (10).

Among studies that have examined *COMT* in relation to mood disorders, while two small Caucasian case-control studies found no consistent evidence of association between *COMT* and unipolar depression (11;12), a large European multi-center study reported significant association of the val allele with early-onset major depression (13). *COMT* was also found to predict onset of depressive episodes following exposure to stressful life events in another large, European sample (14).

Given the potential role of *COMT* in a broad range of internalizing phenotypes, in this study we sought to assess the potential association between *COMT* gene variants and shared genetic

risk across a range of anxiety-related phenotypes in a large, population-based sample. Specifically, we tested the val158met polymorphism as well as other markers that characterize the major allelic variation around the *COMT* locus, together with relevant haplotypes. Also, due to the substantial proportion of reports that were specific to women, we also analyzed our results by sex.

Materials and Methods

Subjects

The subjects in this study derive from the longitudinal population-based Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD) (15;16). All subjects were Caucasian and born in Virginia. Their age (mean, SD, range) at time of last interview was (37.9, 20–58) for males and (36.8, 21–62) for females. Approval of the local Institutional Review Board was obtained prior to the study and informed consent was obtained from all subjects prior to data collection.

Diagnostic Measures

We obtained lifetime psychiatric diagnoses via face-to-face or telephone structured psychiatric interview based on the Structured Clinical Interview for DSM-III-R (SCID) (17). We used DSM-III-R (18) diagnostic criteria to assess lifetime major depression, modified DSM-III-R criteria for lifetime generalized anxiety disorder and panic disorder (19;20), and an adaptation of DSM-III criteria for phobias (21) (22). We included agoraphobia and social phobia in the phenotypic modeling used for this study (see below). Neuroticism was assessed using the 12 items from the short form of the Eysenck Personality Questionnaire (EPQ) (23) via self-report questionnaire.

Sample Selection

As described previously for this sample (24), we have incorporated two novel strategies into our subject selection procedure. First, we have taken advantage of the extant literature that suggests shared genetic susceptibility among neuroticism, the anxiety disorders, and major depression (25–28). Starting with a total of 9270 twin subjects, we used multivariate structural equation modeling to estimate a latent genetic factor for neuroticism that is highly correlated with genetic susceptibility to major depression, generalized anxiety disorder, panic disorder, agoraphobia, and social phobia [see (28) for details]. Like phenotypic factor analysis, the factor derived from this analysis combines information across the correlated measures (phenotypes), but in this case uses shared genetic risk as the basis for this combination. Second, several authors have proposed using extreme phenotypic selection schemes to maximize the difference in information contained in a sample of subjects assessed on continuous measures such as blood pressure or depression scores (29–31). However, unlike these selection schemes based only upon *phenotypic* extremes, the use of a genetically informative sample containing twins allows for the identification of subjects who are at the high and low extremes of *genetic risk* as well, as estimated by the twin pair's score on the above-mentioned genetic factor. Selecting subjects from the extremes of their underlying genetic risk factor should provide a powerful method for detecting genes of small effect expected to contribute to complex genetic phenotypes like psychiatric disorders. One member from each twin pair for whom DNA was available was selected as a case or control based upon scoring above the 80th or below the 20th percentile, respectively, of the genetic factor extracted from the above analysis. This produced a total sample size N= 1128 consisting of 589 cases (350 males, 239 females) and 539 controls (343 males, 196 females), of which 376 (196 males, 180 females) and 752 (497 males, 255 females) were used in stage 1 and stage 2 respectively. We note that this is more than just a selection on neuroticism, as, for example, some pairs score in the upper tail due to high genetic loading on the clinical syndromes but may have relatively modest levels of neuroticism. Overall, the

cases had a mean raw neuroticism score of 6.3 (z-score = 1.04) and had the following frequencies of the target psychiatric illnesses: major depressive disorder (80.1%), generalized anxiety disorder (53.8%), panic disorder (20.5%), agoraphobia (14.1%), and social phobia (17.5%). The controls were free of these five disorders and had a mean raw neuroticism score of 0.55 (z-score = -0.89). These statistics were similar across the two stages.

Genotyping

DNA was extracted from buccal epithelial cells obtained via cytology brushes (32). SNPs were genotyped by the 5' nuclease cleavage assay (also called TaqMan method) (33). Reactions were performed in 96-well plates with 5 µl reaction volume containing 0.25 µl of 20X Assays-on-Demand™ SNP assay mix, 2.5 µl of TaqMan universal PCR master mix, and 5 ng of genomic DNA. Each 96-well plate contains samples for either cases or control, and these are intercalated onto a single 384-well plate within a genotyping run to reduce the risk of batch effects differentially affecting cases versus controls. The conditions for PCR were initial denaturing at 95 °C for 10 minutes, followed by 40 cycles of 92 °C for 15 seconds and 60 °C for 1 minute. After the reaction, fluorescence intensities for reporter 1 (VIC, excitation = 520 ± 10 nm, emission = 550 ± 10 nm) and reporter 2 (FAM, excitation = 490 ± 10 nm, emission = 510 ± 10 nm) were read by the Analyst fluorescence plate reader (LJL Biosystems, Sunnyvale, CA). Genotypes were scored by a Euclidian clustering algorithm developed in our laboratory and checked for deviations from Hardy-Weinberg equilibrium. We performed duplicate genotyping on a subset of plates as a quality control check and for any assays that did not perform optimally.

COMT spans a 27 Kb interval on chromosome 22q11. The functional val158met polymorphism (hereafter referred to as rs4680), occurs in exon 4. Since several studies have questioned whether this polymorphism is the main susceptibility allele in the *COMT* region, we selected other SNP markers in an approximately 32 Kb interval around this gene with the aim to tag the major haplotypes observed in the Caucasian panel used by the HapMap project (34). We used the Tagger module of HAPLOVIEW 3.2 (35) with HapMap Phase II data, specifying aggressive tagging of 2- and 3-marker haplotypes and a threshold of $r^2=0.7$. This provided 8 tag SNPs (including rs4680) that captured the 16 alleles with MAF > 0.05 in that interval with $r^2 > 0.73$. We added SNPs rs737865 and rs165599, given their potential relevance as indicated by prior studies of this gene (7;8), totaling ten SNPs in and around the *COMT* locus for genotyping (see Results).

Statistical Analysis

We used a 2-stage association design in which candidate loci were screened in stage 1, the positive results of which were tested for replication in stage 2. The parameters for this design were calculated using the LGA972 program (36) to achieve 80% power to detect markers that explained 1% of the variance of the liability distribution while controlling the false discovery rate at 0.1 (37). Using a phenotypic extreme selection strategy with thresholds at 20% and 80%, respectively, LGA972 indicated that we needed about 350 subjects in the stage 1 and 1,000 in the stage 2 sample. As described above, we used somewhat smaller numbers than this in stage 2, but our selection was based on extremes of genetic, not phenotypic, factor scores, which should generally provide higher power to detect genetic effects, but this is not simple to estimate. If any of the markers genotyped in stage 1 met the screening p-value threshold of 0.1 or less, they were then also tested in the stage 2 sample.

We used Pearson's chi-squared tests to test for allelic or genotypic differences by marker between cases and controls, separately by stage in order to check for consistency of results across the two stages. We used the program PEDSTATS (38) to test for Hardy-Weinberg equilibrium (HWE) violations for each marker. We used HAPLOVIEW 3.2 (35) to characterize

linkage disequilibrium (LD) between the markers in our sample. Case-control haplotypic association analyses were performed with the Cocaphase module of the UNPHASED program, version 2.4 (39). UNPHASED uses the expectation-maximization algorithm (40) to estimate the haplotypes and their frequencies. We used the program PHASE, version 2.1 (41;42), to reconstruct most likely multi-marker haplotypes for each subject for use in post-hoc regression analyses when there were not 1-to-1 relationships between marker genotypes and haplotypes. We note that, despite the suggestive name of the software, we can only estimate haplotypes of unknown phase, given the case-control nature of our data. We performed each of these analyses for men and women together and separately

For those markers or haplotypes that were consistently significant in each stage, we performed an overall analysis by combining data from both stages. A complication is that markers are selected for genotyping in stage 2 conditional upon their p-values in stage 1, so the test in stage 2 for any particular marker is not independent from that in stage 1. Assuming the conventional chi-square distribution for the test statistic would result in a considerable increase in the number of false discoveries (43;44). To perform accurate tests on the combined data, we therefore used a different test statistic distribution that we derived previously (45;46).

The risk of false discoveries is considerable in candidate gene studies (47–49). To better assess this risk, we estimated the q-value for each marker genotyped in stage 2, which can be interpreted as the probability that a marker identified as significant is a false discovery (50–52). To estimate the q-values, one needs to know the prior probability that the marker has an effect as well as the effect size of the marker. Because the prior probability cannot be estimated reliably when only a relatively few markers are genotyped, we assumed a range of possible values for our calculations. Also, markers that, due to sampling fluctuations, have a larger effect size are more likely to be selected as significant in stage 1. To estimate the effect size (odds ratio), we used data from stage 2 only, because the estimate tends to approach the true effect size in an independent sample (53).

Results

Association Testing

The genotype and allele frequencies and results of chi-squared association tests for the ten *COMT* markers genotyped in stage 1 are listed in Table 2. P-values are shown for the entire stage 1 sample and broken down by sex to indicate from which group significance may derive. In order to conserve space and simplify the table, the genotype and allele frequencies are only shown for the combination of males and females (the values broken down by sex are available upon request). All markers except three were in HWE: markers 5 and 10 showed modest deviations from HWE ($p=0.002$ and $p=.025$, respectively) in cases only, while marker 7 showed more severe deviation ($p=0.0006$) in both cases and controls. Figure 1 depicts the relative positions of these markers with respect to the intron-exon structure of the *COMT* gene.

Linkage disequilibrium (LD) information for these markers in our stage 1 sample is also provided in Figure 1 (using D') and Supplementary Table 1 (both D' and r^2). To better understand the LD structure, we constructed haplotype blocks using the default Confidence Interval procedure (54) in HAPLOVIEW 3.2. As indicated in Figure 1, pairs of markers (1, 2), (5,6), and (9,10) are in high LD, with the latter two pairs being part of haplotype blocks. This is roughly consistent with data from HapMap. We note that LD is quite modest across the *COMT* gene overall. This would generally disfavor performing haplotype association tests for widely-spaced markers across the gene. However, prior analyses in schizophrenia (8) and personality (7) (among other phenotypes) have produced significant findings using combinations of rs4680 with markers 2 and 10 in this locus. Based upon this, we created haplotypes from relevant marker combinations.

Markers 4, 7, and 9 met threshold criteria of allelic p -value < 0.1 in our stage 1 sample; we genotyped these plus markers 2 and 10 in stage 2 (see Table 3). We chose these latter two markers to analyze relevant haplotypes in both stages implicated in prior studies. (Note: while marker 3 displayed significant association in the genotypic analysis in stage 1, particularly for females, this differed from the results of the allelic analysis. This, together with the results of exploratory analyses that suggested that this marker does not contribute to haplotypic association, led us to decide not to genotype it in stage 2.) Comparing Tables 2 and Table 3, there are no clearly consistent single marker associations across stages. While marker 7 showed marginal association in stage 2, it continued to suffer from severe deviations from HWE in the total sample ($p=1.3e-5$); the other four markers did not show HWE deviations when the entire sample was considered. Therefore, we excluded this marker from further analyses.

In Table 4, we present the results, by stage, of haplotype association tests for several relevant marker combinations as calculated using the Cocophase module of UNPHASED. The haplotypes constructed from markers (2-4-10) correspond to those analyzed by Shifman and colleagues (8) for schizophrenia and Stein and colleagues for personality (7); it did not produce a pattern of association that was replicated across stages. However, closer inspection of these results suggested that the G-A haplotype of the simpler combination of markers 4 and 10 within that three-marker combination was driving associations that were seen, particularly in women. Marker 2, further away from, and in low LD with, the other two markers, appeared to be diluting the signal. This was supported by the stronger, more consistent association seen with the G-A haplotype from markers 4 and 10, which is also more common than the three-marker haplotypes examined. This association is confined to the women.

Based upon these findings, we sought to analyze the effects of our best candidate markers and haplotypes in the combined stage 1 + stage 2 female sample. For this purpose, we chose marker 4 (rs4680) due to its relevance from prior studies, and our G-A haplotype from markers 4 and 10. We used the program PHASE, version 2.1 (41;42), to reconstruct these two-marker haplotypes for all individuals in our sample. In order to maximize confidence in the estimated haplotypes, we used the most likely pairs of haplotypes for each subject and discarded data from subjects if haplotype probabilities did not exceed 0.8.

Table 5 displays the p -values for female subjects pooled across both stages and the stage 2 case-control odds ratios for marker 4 (rs4680) and the G-A haplotype. We estimated a roughly 24% increase in risk associated with the G (val) allele of marker 4 and almost double the risk associated with the G-A haplotype from markers 4 and 10. Table 5 also displays the estimated false-discovery rate q , that is, the global probability that the combined results for each particular marker or haplotype occurred purely by chance, as a function of the assumed prior probability of true discovery, p_0 . While q -values are above 50% for marker 4, those for the G-A haplotype are all below 6%. This suggests that, when the corresponding p -value ($1.97e-5$) is used to declare significance, the expected proportion of false discoveries among significant tests would be 6% or less.

In post-hoc analyses, we explored whether these findings for *COMT*, based upon individual differences in genetic factor scores, were a result of associations with specific phenotypes within our total sample. Using Cochran-Mantel-Haenszel tests in the FREQ procedure of SAS (55), we detected significant associations (non-zero correlation) between marker 4 and the G-A haplotype and most of our measured phenotypes, as shown in Table 6. For simplicity, we only display the results for women, as no associations were observed for men. We note that these analyses extend beyond our original hypotheses and do not control for such factors as multiple testing or correlated phenotypes. The relationship between mean N score and number of copies of the G-A haplotype in females is graphed in Figure 2.

Population Stratification

As a potential concern for any case-control association study, we explored the possibility that these results were obtained spuriously due to population stratification using three methods. First, using self-reported ancestry data from this entirely Caucasian sample, we did not detect any evidence of ethnic background differences between cases and controls. Second, using a set of 24 unlinked markers chosen for convenience from experiments on other candidate loci, the software STRUCTURE (56) found no significant genetic subpopulations. Finally, using the method of Genomic Control (57) on the same 24 unlinked markers, we found no evidence of variance inflation that could be attributed to stratification. In addition to these investigations in the current sample, Sullivan et al (58) found no evidence for stratification using 16 unlinked microsatellite markers in a case-control study of nicotine dependence in a different subset of our twin sample (n=900).

Discussion

In this study, we sought to test whether the *COMT* gene is associated with susceptibility to human anxiety spectrum phenotypes, including neuroticism, a range of anxiety disorders, and major depression. This susceptibility was indexed by a latent genetic factor common to these phenotypes, the score of which we derived from multivariate twin modeling and subsequently used to select subjects at the extremes of genetic risk. We entered the resulting sample of 589 cases and 539 controls into a two-stage association study in which markers from the candidate locus were screened in stage 1, the positive results of which were tested for replication in stage 2. Because prior studies highlight the potential importance of analyzing haplotypes across this gene, we tested several markers in addition to the functional val158met polymorphism (rs4680).

Out of a total of ten markers tested in the *COMT* gene, three met the threshold criterion in stage 1 of $p < 0.1$ for genotyping in stage 2. These three, plus two others selected based upon haplotype analyses from other studies, were then genotyped in the stage 2 sample. While there were no consistent associations seen across the two stages for individual markers, rs4680 showed marginally significant association in women when data from both stages were combined. The G-A haplotype formed from the G (val) allele of marker rs4680 together with the A allele of rs165599 showed significant association in each stage and in the entire female sample ($p=1.97 \times 10^{-5}$). Further, this haplotype more significantly predicted, than did rs4680 alone, each of the specific psychiatric phenotypes relevant for our study (again, in women only), including neuroticism ($p=1 \times 10^{-5}$), major depression ($p=2 \times 10^{-4}$), and several anxiety disorders. We tested for, and could not detect, any evidence that these were spurious association signals due to population stratification. However, this latter analysis was limited to only 24 markers, which may be insufficient to detect modest levels of stratification (59).

Declaring a positive association in a candidate gene study is not without controversy (60). While no individual marker, including rs4680, displayed consistent association across both stages, several haplotypes did, specifically in women. While this is not a formal replication by recent standards (60), since both stages derive from the same overall sample of twins, it nonetheless provided justification for jointly analyzing data across the two stages to maximize power (61). A naïve application of Bonferroni correction (i.e., dividing a significance threshold of $p=0.05$ by the total number of tests performed) may not be appropriate here, given the non-independence between tests of markers in LD with each other and overlapping haplotypes constructed from them. Also, in general there are practical and theoretical limitations to the Bonferroni correction (44). Therefore, we estimated the probability that our *a priori* candidate polymorphism (val158met) and our best haplotype were false discoveries (q-values). Examining Table 5, we see that, even though rs4680 showed association at $p=0.0094$ level of significance, this also has high probability of being a false discovery. However, the G-A

haplotype formed from markers rs460 and rs165599 not only showed highly significant association in women, but our q-value estimates suggest that this is unlikely a false discovery.

The fact that our identified high-risk haplotype had significant allelic differences in our original sample selected for shared genetic risk for internalizing phenotypes and significantly predicted each of the individual phenotypes themselves suggests that *COMT*, or another locus in LD with this haplotype, acts as a broad psychiatric risk factor. Consistent with studies in other phenotypes, *COMT* haplotypes containing marker rs4680 confer a greater effect on risk than the functional val158met polymorphism alone, although exactly which allele corresponds to increased risk appears to depend upon phenotype studied and ethnic makeup of the sample, as reviewed in the Introduction. Our high-risk haplotype contains the G (val) allele of rs4680. This is consistent with prior studies of panic disorder in Caucasian samples (10) as well as a large European study of early-onset major depressive disorder (13). However, this appears to be somewhat at odds with a number of prior studies of neuroticism and other anxiety-related traits and symptoms (see Table 1). While several of the smaller studies reported marginally significant associations with the met allele or met-met genotype, the two largest studies either found no association with this locus (62) or association with the opposite (val) allele (4). Among those studies, only Stein and colleagues examined the effects of markers other than rs4680 (7). In a mixed ethnic sample of 497 college students, they tested the three markers (rs737865, rs4680, and rs165599 – corresponding to our markers 2, 4, and 10) and related haplotypes previously found to be highly associated with schizophrenia in a sample of Ashkenzi Jews (8). They found marginally significant associations of the met-met genotype with low extroversion (a trait related to social phobia) and high neuroticism in their female subjects and variously associated three-marker haplotypes depending upon whether extroversion and neuroticism were analyzed via median split of their sample or as continuous traits. Specifically, while their strongest reported association was between the G-A-A haplotype and dichotomous neuroticism in women ($p=0.005$), associations were also seen in their results tables for haplotypes containing the G-A alleles of markers 4 and 10 and both phenotypes. For comparison, we note that Shifman et al. reported that the G (val) allele was modestly associated with schizophrenia in men, with the strongest associations from the three-marker haplotype G-G-G (8).

Given the approximately two-fold increased risk for anxiety and depressive disorders in women compared with men, our identification of a haplotype that broadly increases risk for internalizing disorders in women only is intriguing. However, the mechanism by which this haplotype increases risk in women but not men is yet to be discerned. In our sample, men and women did not, on average, significantly differ in the frequencies of this high-risk haplotype. One hypothesis is that differing levels of estrogenic hormones between women and men interact with this haplotype to differentially affect risk. Multi-marker *COMT* haplotypes have been shown to modulate expression of this gene in the brain (6), possibly in a sex-specific fashion (63). Furthermore, the *COMT* gene has an estrogenic response element in its promoter region, so certain haplotypes containing this could produce differential expression of *COMT* depending upon estrogen levels (64). As a key enzyme in the conjugation of catecholestrogens (65), *COMT* genotype also affects circulating levels of estrogen (66), suggesting a complex bidirectional interaction between estrogenic hormones and *COMT* haplotypes which could, presumably, impact psychiatric risk in a sex-specific manner.

In conclusion, we have identified genetic variations in the *COMT* gene that significantly associate with a range of internalizing psychiatric phenotypes. In particular, in agreement with prior studies that have examined multiple markers across this locus, the val158met polymorphism alone is unable to fully explain this association. Rather, haplotype analyses suggest that variations in or around *COMT* in LD with this polymorphism increase susceptibility for women, but exactly where or on what level these variations contribute to this

risk remains unclear. Future studies of *COMT* would thus benefit from including other markers in addition to the val158met polymorphism and performing sex-specific analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Reference List

1. Craddock N, Owen MJ, O'donovan MC. The Catechol-O-Methyl Transferase (COMT) Gene As a Candidate for Psychiatric Phenotypes: Evidence and Lessons. *Mol.Psychiatry* 2006;11(5):446–458. [PubMed: 16505837]
2. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human Catechol-O-Methyltransferase Pharmacogenetics: Description of a Functional Polymorphism and Its Potential Application to Neuropsychiatric Disorders. *Pharmacogenetics* 1996;6(3):243–250. [PubMed: 8807664]
3. Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, Karayiorgou M. Catechol-O-Methyltransferase-Deficient Mice Exhibit Sexually Dimorphic Changes in Catecholamine Levels and Behavior. *Proc.Natl.Acad.Sci.U.S.A* 1998 Aug 18;95(17):9991–9996. [PubMed: 9707588]
4. McGrath M, Kawachi I, Ascherio A, Colditz GA, Hunter DJ, De, Vivo I. Association Between Catechol-O-Methyltransferase and Phobic Anxiety. *Am.J.Psychiatry* 2004;161(9):1703–1705. [PubMed: 15337664]
5. Kim SJ, Kim YS, Kim SY, Lee HS, Kim CH. An Association Study of Catechol-O-Methyltransferase and Monoamine Oxidase A Polymorphisms and Personality Traits in Koreans. *Neurosci.Lett* 2006 Jun 19;401(1–2):154–158. [PubMed: 16584839]
6. Bray NJ, Buckland PR, Williams NM, Williams HJ, Norton N, Owen MJ, O'donovan MC. A Haplotype Implicated in Schizophrenia Susceptibility Is Associated With Reduced COMT Expression in Human Brain. *Am.J.Hum.Genet* 2003;73(1):152–161. [PubMed: 12802784]
7. Stein MB, Fallin MD, Schork NJ, Gelernter J. COMT Polymorphisms and Anxiety-Related Personality Traits. *Neuropsychopharmacology* 2005;30(11):2092–2102. [PubMed: 15956988]
8. Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, Weizman A, Reznik I, Spivak B, Grisaru N, Karp L, Schiffer R, Kotler M, Strous RD, Swartz-Vanetik M, Knobler HY, Shinar E, Beckmann JS, Yakir B, Risch N, Zak NB, Darvasi A. A Highly Significant Association Between a COMT Haplotype and Schizophrenia. *Am.J.Hum.Genet* 2002;71(6):1296–1302. [PubMed: 12402217]
9. Hamilton SP, Slager SL, Heiman GA, Deng Z, Haghighi F, Klein DF, Hodge SE, Weissman MM, Fyer AJ, Knowles JA. Evidence for a Susceptibility Locus for Panic Disorder Near the Catechol-O-Methyltransferase Gene on Chromosome 22. *Biol.Psychiatry* 2002 Apr 1;51(7):591–601. [PubMed: 11950461]
10. Domschke K, Deckert J, O'donovan MC, Glatt SJ. Meta-Analysis of COMT Val158met in Panic Disorder: Ethnic Heterogeneity and Gender Specificity. *Am.J.Med.Genet.B Neuropsychiatr.Genet* 2007 Jul 5;144(5):667–673. [PubMed: 17357147]
11. Frisch A, Postilnick D, Rockah R, Michaelovsky E, Postilnick S, Birman E, Laor N, Rauchverger B, Kreinin A, Poyurovsky M, Schneidman M, Modai I, Weizman R. Association of Unipolar Major Depressive Disorder With Genes of the Serotonergic and Dopaminergic Pathways. *Mol.Psychiatry* 1999;4(4):389–392. [PubMed: 10483058]

12. Kunugi H, Vallada HP, Hoda F, Kirov G, Gill M, Aitchison KJ, Ball D, Arranz MJ, Murray RM, Collier DA. No Evidence for an Association of Affective Disorders With High- or Low-Activity Allele of Catechol-o-Methyltransferase Gene. *Biol.Psychiatry* 1997 Aug 15;42(4):282–285. [PubMed: 9270905]
13. Massat I, Souery D, Del Favero J, Nothen M, Blackwood D, Muir W, Kaneva R, Serretti A, Lorenzi C, Rietschel M, Milanova V, Papadimitriou GN, Dikeos D, Van Broekhoven C, Mendlewicz J. Association Between COMT (Val158Met) Functional Polymorphism and Early Onset in Patients With Major Depressive Disorder in a European Multicenter Genetic Association Study. *Mol.Psychiatry* 2005;10(6):598–605. [PubMed: 15583702]
14. Mandelli L, Serretti A, Marino E, Pirovano A, Calati R, Colombo C. Interaction Between Serotonin Transporter Gene, Catechol- O -Methyltransferase Gene and Stressful Life Events in Mood Disorders. *Int.J.Neuropsychopharmacol* 2006 Jun 7;:1–11. [PubMed: 16045810]
15. Kendler KS, Prescott CA. A Population-Based Twin Study of Lifetime Major Depression in Men and Women. *Arch.Gen.Psychiatry* 1999;56(1):39–44. [PubMed: 9892254]
16. Kendler, KS.; Prescott, CA. *Genes, Environment, and Psychopathology: Understanding the Causes of Psychiatric and Substance Use Disorders*. New York: Guilford Press; 2006.
17. Spitzer, RL.; Williams, JBW. *Structured Clinical Interview for DSM-III-R (SCID)*. New York: Biometrics Research Department, New York State Psychiatric Institute; 1985.
18. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. Revised Third Edition. Washington, DC: American Psychiatric Association; 1987.
19. Hettema JM, Prescott CA, Kendler KS. A Population-Based Twin Study of Generalized Anxiety Disorder in Men and Women. *J.Nerv.Ment.Dis* 2001;189(7):413–420. [PubMed: 11504317]
20. Kendler KS, Gardner CO, Prescott CA. Panic Syndromes in a Population-Based Sample of Male and Female Twins. *Psychol.Med* 2001;31(6):989–1000. [PubMed: 11513384]
21. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. Third Edition. Washington, DC: American Psychiatric Association; 1980.
22. Kendler KS, Myers J, Prescott CA, Neale MC. The Genetic Epidemiology of Irrational Fears and Phobias in Men. *Arch.Gen.Psychiatry* 2001;58(3):257–265. [PubMed: 11231833]
23. Eysenck, HJ.; Eysenck, SBG. *Manual of the Eysenck Personality Questionnaire*. London: Hodder and Stoughton; 1975.
24. Hettema JM, An SS, Neale MC, Bukszar J, van den Oord EJ, Kendler KS, Chen X. Association Between Glutamic Acid Decarboxylase Genes and Anxiety Disorders, Major Depression, and Neuroticism. *Mol.Psychiatry* 2006;11(8):752–762. [PubMed: 16718280]
25. Middeldorp CM, Cath DC, Van Dyck R, Boomsma DI. The Co-Morbidity of Anxiety and Depression in the Perspective of Genetic Epidemiology. A Review of Twin and Family Studies. *Psychol.Med* 2005;35(5):611–624. [PubMed: 15918338]
26. Jardine R, Martin NG, Henderson AS. Genetic Covariation Between Neuroticism and the Symptoms of Anxiety and Depression. *Genet.Epidemiol* 1984;1(2):89–107. [PubMed: 6544237]
27. Scherrer JF, True WR, Xian H, Lyons MJ, Eisen SA, Goldberg J, Lin N, Tsuang MT. Evidence for Genetic Influences Common and Specific to Symptoms of Generalized Anxiety and Panic. *J Affect.Disord* 2000;57(1–3):25–35. [PubMed: 10708813]
28. Hettema JM, Neale MC, Myers JM, Prescott CA, Kendler KS. A Population-Based Twin Study of the Relationship Between Neuroticism and Internalizing Disorders. *Am.J.Psychiatry* 2006;163(5):857–864. [PubMed: 16648327]
29. Van Gestel S, Houwing-Duistermaat JJ, Adolfsson R, van Duijn CM, Van Broeckhoven C. Power of Selective Genotyping in Genetic Association Analyses of Quantitative Traits. *Behav.Genet* 2000;30(2):141–146. [PubMed: 10979604]
30. van den Oord EJCG. A Comparison Between Different Designs and Tests to Detect QTLs in Association Studies. *Behavior Genetics* 1999;29(4):245–256.
31. Schork NJ, Nath SK, Fallin D, Chakravarti A. Linkage Disequilibrium Analysis of Biallelic DNA Markers, Human Quantitative Trait Loci, and Threshold-Defined Case and Control Subjects. *Am.J.Hum.Genet* 2000;67(5):1208–1218. [PubMed: 11032785]
32. Straub RE, Sullivan PF, Ma Y, Myakishev MV, Harris-Kerr C, Wormley B, Kadambi B, Sadek H, Silverman MA, Webb BT, Neale MC, Bulik CM, Joyce PR, Kendler KS. Susceptibility Genes for

Nicotine Dependence: a Genome Scan and Followup in an Independent Sample Suggest That Regions on Chromosomes 2, 4, 10, 16, 17 and 18 Merit Further Study. *Mol.Psychiatry* 1999;4(2):129–44. [PubMed: 10208445]

33. Livak KJ. Allelic Discrimination Using Fluorogenic Probes and the 5' Nuclease Assay. *Genet.Anal* 1999;14(5–6):143–149. [PubMed: 10084106]
34. The International HapMap Project. *Nature* 2003 Dec 18;426(6968):789–796. [PubMed: 14685227]
35. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and Visualization of LD and Haplotype Maps. *Bioinformatics* 2005 Jan 15;21(2):263–265. [PubMed: 15297300]
36. Robles JR, van den Oord EJ. Lga972: a Cross-Platform Application for Optimizing LD Studies Using a Genetic Algorithm. *Bioinformatics* 2004 Nov 22;20(17):3244–3245. [PubMed: 15180931]
37. van den Oord EJ, Sullivan PF. False Discoveries and Models for Gene Discovery. *Trends Genet* 2003;19(10):537–542. [PubMed: 14550627]
38. Wigginton JE, Abecasis GR. PEDSTATS: Descriptive Statistics, Graphics and Quality Assessment for Gene Mapping Data. *Bioinformatics* 2005 Aug 15;21(16):3445–3447. [PubMed: 15947021]
39. Dudbridge F. Pedigree Disequilibrium Tests for Multilocus Haplotypes. *Genet.Epidemiol* 2003;25(2):115–121. [PubMed: 12916020]
40. Excoffier L, Slatkin M. Maximum-Likelihood Estimation of Molecular Haplotype Frequencies in a Diploid Population. *Mol.Biol.Evol* 1995;12(5):921–927. [PubMed: 7476138]
41. Stephens M, Donnelly P. A Comparison of Bayesian Methods for Haplotype Reconstruction From Population Genotype Data. *Am.J.Hum.Genet* 2003;73(5):1162–1169. [PubMed: 14574645]
42. Stephens M, Smith NJ, Donnelly P. A New Statistical Method for Haplotype Reconstruction From Population Data. *Am.J.Hum.Genet* 2001;68(4):978–989. [PubMed: 11254454]
43. Lowe CE, Cooper JD, Chapman JM, Barratt BJ, Twells RC, Green EA, Savage DA, Guja C, Ionescu-Tirgoviste C, Tuomilehto-Wolf E, Tuomilehto J, Todd JA, Clayton DG. Cost-Effective Analysis of Candidate Genes Using HtSNPs: a Staged Approach. *Genes Immun* 2004;5(4):301–305. [PubMed: 15029236]
44. van den Oord EJ, Sullivan PF. A Framework for Controlling False Discovery Rates and Minimizing the Amount of Genotyping in the Search for Disease Mutations. *Hum.Hered* 2003;56(4):188–199. [PubMed: 15031620]
45. Bukszar J, van den Oord EJ. Accurate and Efficient Power Calculations for 2 x m Tables in Unmatched Case-Control Designs. *Stat.Med* 2006 Aug 15;25(15):2632–2646. [PubMed: 16025555]
46. Bukszar J, van den Oord EJ. Optimization of Two-Stage Genetic Designs Where Data Are Combined Using an Accurate and Efficient Approximation for Pearson's Statistic. *Biometrics* 2006;62(4):1132–1137. [PubMed: 17156288]
47. Colhoun HM, McKeigue PM, Davey Smith G. Problems of Reporting Genetic Associations With Complex Outcomes. *Lancet* 2003 Mar 8;361(9360):865–872. [PubMed: 12642066]
48. Freimer N, Sabatti C. The Use of Pedigree, Sib-Pair and Association Studies of Common Diseases for Genetic Mapping and Epidemiology. *Nat.Genet* 2004;36(10):1045–1051. [PubMed: 15454942]
49. Van den Oord EJCG. Controlling False Discoveries in Candidate Gene Studies. *Molecular Psychiatry* 2005;10:230–231. [PubMed: 15738930]
50. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the Probability That a Positive Report Is False: an Approach for Molecular Epidemiology Studies. *J.Natl.Cancer Inst* 2004 Mar 17;96(6):434–442. [PubMed: 15026468]
51. Storey JD, Tibshirani R. Statistical Significance for Genomewide Studies. *Proc.Natl.Acad.Sci.U.S.A* 2003 Aug 5;100(16):9440–9445. [PubMed: 12883005]
52. Van den Oord EJCG, Sullivan PF. False Discoveries and Models for Gene Discovery. *Trends in Genetics* 2003;19:537–542. [PubMed: 14550627]
53. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication Validity of Genetic Association Studies. *Nat Genet* 2001;29(3):306–309. [PubMed: 11600885]
54. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The Structure of Haplotype Blocks in the Human Genome. *Science* 2002 Jun 21;296(5576):2225–2229. [PubMed: 12029063]

55. SAS Institute. SAS/STAT Software: Version 8. Cary, NC: SAS Institute Inc; 1999.
56. Pritchard JK, Stephens M, Donnelly P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 2000;155(2):945–959. [PubMed: 10835412]
57. Devlin B, Roeder K. Genomic Control for Association Studies. *Biometrics* 1999;55(4):997–1004. [PubMed: 11315092]
58. Sullivan PF, Neale MC, Silverman MA, Harris-Kerr C, Myakishev MV, Wormley B, Webb BT, Ma Y, Kendler KS, Straub RE. An Association Study of DRD5 With Smoking Initiation and Progression to Nicotine Dependence. *Am.J.Med.Genet* 2001 Apr 8;105(3):259–265. [PubMed: 11353446]
59. Freedman ML, Reich D, Penney KL, McDonald GJ, Mignault AA, Patterson N, Gabriel SB, Topol EJ, Smoller JW, Pato CN, Pato MT, Petryshen TL, Kolonel LN, Lander ES, Sklar P, Henderson B, Hirschhorn JN, Altshuler D. Assessing the Impact of Population Stratification on Genetic Association Studies. *Nat.Genet* 2004;36(4):388–393. [PubMed: 15052270]
60. DeLisi, LE.; Faraone, SV. *Am.J.Med.Genet.B Neuropsychiatr.Genet.* 141. 2006 Jun 5. When Is a "Positive" Association Truly a "Positive" in Psychiatric Genetics? A Commentary Based on Issues Debated at the World Congress of Psychiatric Genetics, Boston, October 12–18, 2005; p. 319–322.
61. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint Analysis Is More Efficient Than Replication-Based Analysis for Two-Stage Genome-Wide Association Studies. *Nat.Genet* 2006;38(2):209–213. [PubMed: 16415888]
62. Henderson AS, Korten AE, Jorm AF, Jacomb PA, Christensen H, Rodgers B, Tan X, Eastaer S. COMT and DRD3 Polymorphisms, Environmental Exposures, and Personality Traits Related to Common Mental Disorders. *Am.J.Med.Genet* 2000 Feb 7;96(1):102–107. [PubMed: 10686561]
63. Dempster EL, Mill J, Craig IW, Collier DA. The Quantification of COMT mRNA in Post Mortem Cerebellum Tissue: Diagnosis, Genotype, Methylation and Expression. *BMC.Med.Genet* 2006;7(10)
64. Bourdeau V, Deschenes J, Metivier R, Nagai Y, Nguyen D, Bretschneider N, Gannon F, White JH, Mader S. Genome-Wide Identification of High-Affinity Estrogen Response Elements in Human and Mouse. *Mol.Endocrinol* 2004;18(6):1411–1427. [PubMed: 15001666]
65. Liehr JG, Roy D. Free Radical Generation by Redox Cycling of Estrogens. *Free Radic.Biol.Med* 1990;8(4):415–423. [PubMed: 2199344]
66. Worda C, Sator MO, Schneeberger C, Jantschev T, Ferlitsch K, Huber JC. Influence of the Catechol-O-Methyltransferase (COMT) Codon 158 Polymorphism on Estrogen Levels in Women. *Hum.Reprod* 2003;18(2):262–266. [PubMed: 12571159]
67. Enoch MA, Xu K, Ferro E, Harris CR, Goldman D. Genetic Origins of Anxiety in Women: a Role for a Functional Catechol-O-Methyltransferase Polymorphism. *Psychiatr.Genet* 2003;13(1):33–41. [PubMed: 12605099]
68. Olsson CA, Anney RJ, Lotfi-Miri M, Byrnes GB, Williamson R, Patton GC. Association Between the COMT Val158Met Polymorphism and Propensity to Anxiety in an Australian Population-Based Longitudinal Study of Adolescent Health. *Psychiatr.Genet* 2005;15(2):109–115. [PubMed: 15900225]
69. Eley TC, Tahir E, Angleitner A, Harriss K, McClay J, Plomin R, Riemann R, Spinath F, Craig I. Association Analysis of MAOA and COMT With Neuroticism Assessed by Peers. *Am.J.Med.Genet.B Neuropsychiatr.Genet* 2003 Jul 1;120(1):90–96. [PubMed: 12815746]
70. Ishii G, Suzuki A, Oshino S, Shiraishi H, Matsumoto Y, Otani K, Goto K. Association Study of Catechol-O-Methyltransferase Val158Met Polymorphism With Personality Traits in Japanese Healthy Volunteers. *Eur.Psychiatry* 2007;22(7):462–465. [PubMed: 17761405]
71. Hashimoto R, Noguchi H, Hori H, Ohi K, Yasuda Y, Takeda M, Kunugi H. A Possible Association Between the Val158Met Polymorphism of the Catechol-O-Methyl Transferase Gene and the Personality Trait of Harm Avoidance in Japanese Healthy Subjects. *Neurosci.Lett* 2007 Nov 20;428(1):17–20. [PubMed: 17945417]

Figure 1.

Ten SNP markers genotyped across the *COMT* locus, with exons and untranslated regions (UTRs) as indicated. Linkage disequilibrium data (D') and haplotype block pattern from HAPLOVIEW are displayed for these markers in the stage 1 sample.

Figure 2.

Graph of the relationship between mean neuroticism (N) score and number of copies of two-marker (rs4680, rs165599) G-A risk haplotype identified in *COMT* gene.

Table 1

Genetic Association Studies of *COMT* val158met Polymorphism (rs4680) with Anxiety Symptoms or Anxiety-related Traits

Study	Relevant Phenotype	Sample N, ethnicity	Associated phenotype and allele or genotype (p-value)	Comments
Enoch (2003) (67)	TPQ HA, EPQ N	149 Caucasian	HA: Met-Met (p~0.03)	(1) Associations limited to females (2) Trend for association of Met-Met with N
		252 NA	HA: Met-Met (p=0.013)	
Olsson (2005) (68)	CIS-R anxiety scales, NEO N	962 Caucasian	"Episodic anxiety": Met-Met (p=0.02)	(1) Associations limited to females (2) No association with N or "generalized anxiety"
Eley (2003) (69)	NEO N	119 Caucasian	N: Met allele (p=0.05)	(1) Associations limited to females
Henderson (2000) (62)	EPQ N/E/P, BI, others	2,327 Caucasian	No association with any measures	(1) <i>COMT</i> only tested in first stage sample (N=848) (2) No reported analyses by gender
McGrath (2004) (4)	phobic anxiety scale	1,234 Caucasian	Phobia Scale: Val-Val (p=0.01)	Entirely female sample
Stein (2005) (7)	NEO N/E	497 Mixed	Low E: Met-Met (p=0.02)	(1) Associations limited to females (2) Three-marker haplotypes associated with N/E
Kim (2006) (5)	TCI HA	286 Korean	HA: Val-Val (p=0.003)	(1) Associations limited to females
Ishii (2007) (70)	TCI HA	478 Japanese	HA: Met-Met (p=.059)	(1) Trend level association in females
Hashimoto (2007) (71)	TCI HA	139 Japanese	HA: Met allele (p=0.013)	(1) Sex-specific analyses were non-significant

Abbreviations: TPQ – Tridimensional Personality Questionnaire; TCI – Temperament and Character Inventory; HA – harm avoidance; EPQ-Eysenck's Personality Questionnaire; N – neuroticism; NA - Native American; CIS-R - Clinical Interview Schedule-Revised; NEO – NEO personality inventory; E – extroversion; P- Psychoticism; BI – behavioral inhibition

Table 2

COMT Individual Marker Association Results for Stage 1 (N=188 cases, 188 controls) for males [M] (N=196), females [F] (N=180), and together [All]. Bolded text indicates p-values that met the stage 1 screening threshold $p < 0.1$.

Marker	Marker ID (dbSNP)	Alleles (major)	Group	Genotypes (%)			Genotypic p-Value			Alleles (%)			Allelic p-Value	
				A1/A1	A1/A2	A2/A2	All	M	F	A1	A2	All	M	F
1	rs2020917	T/C (C)	Cases	52.4	40.1	7.5	0.73	0.22	0.51	72.5	27.5	0.43	0.24	0.96
			Controls	56.4	37.2	6.4				75.0	25.0			
2	rs737865	A/G (A)	Cases	52.7	40.9	6.4	0.53	0.31	0.66	73.1	26.9	0.27	0.23	0.72
			Controls	58.1	37.0	4.9				76.6	23.4			
3	rs740603	A/G (A)	Cases	28.6	48.7	22.7	0.015	0.16	0.045	53.0	47.0	0.30	0.18	0.91
			Controls	17.8	62.7	19.5				49.2	50.8			
4	rs4680	A/G (A)	Cases	25.5	53.2	21.3	0.12	0.26	0.040	52.2	47.9	0.048	0.48	0.034
			Controls	34.1	50.5	15.4				59.3	40.7			
5	rs4646316	T/C (C)	Cases	59.6	29.8	10.6	0.33	0.48	0.44	74.5	25.5	0.23	0.71	0.18
			Controls	62.8	30.8	6.4				78.2	21.8			
6	rs165774	A/G (A)	Cases	45.4	45.3	9.3	0.96	0.64	0.78	68.0	32.0	0.79	0.61	0.86
			Controls	46.9	44.1	9.0				68.9	31.1			
7	rs174696	T/C (C)	Cases	66.1	25.3	8.6	0.11	0.15	0.61	78.8	21.2	0.065	0.078	0.44
			Controls	55.7	34.6	9.7				73.0	27.0			
8	rs174699	T/C (C)	Cases	88.8	11.2	0.0	0.90	0.64	0.46	94.4	5.6	0.90	0.65	0.47
			Controls	89.2	10.8	0.0				94.6	5.4			
9	rs9332377	T/C (C)	Cases	68.6	28.7	2.7	0.30	0.13	0.30	83.0	17.0	0.15	0.056	1.0
			Controls	74.5	24.5	1.0				86.7	13.3			
10	rs165599	A/G (A)	Cases	48.1	46.5	5.4	0.81	0.95	0.82	71.4	28.6	0.66	0.91	0.59
			Controls	46.8	46.3	6.9				69.9	30.1			

Table 3

COMT Individual Marker Association Results for Stage 2 (N=401 cases, 351 controls) for males [M] (N=497), females [F] (N=255), and together [All]. Bolded text denotes p-values <0.05.

Marker	Marker ID (dbSNP)	Alleles (major)	Group	Genotypes (%)			Genotypic p-Value			Alleles (%)			Allelic p-Value	
				A1/A1	A1/A2	A2/A2	All	M	F	A1	A2	All	M	F
2	rs737865	A/G (A)	Cases	49.2	43.0	7.8	0.14	0.43	0.23	70.7	29.3	0.25	0.38	0.49
			Controls	55.5	35.8	8.7				73.4	26.6			
4	rs4680	A/G (A)	Cases	26.6	48.0	25.4	0.89	0.74	0.35	50.6	49.4	0.63	0.77	0.23
			Controls	28.0	47.7	24.3				51.9	48.1			
7	rs174696	T/C (T)	Cases	64.4	30.0	5.6	0.26	0.58	0.23	79.4	20.6	0.099	0.42	0.072
			Controls	59.8	31.9	8.3				75.7	24.3			
9	rs9332377	T/C (T)	Cases	72.4	25.3	2.3	0.22	0.028	0.51	85.0	15.0	0.95	0.49	0.25
			Controls	74.0	21.8	4.2				84.9	15.1			
10	rs165599	A/G (A)	Cases	53.8	37.2	9.0	0.14	0.44	0.19	72.4	27.6	0.042	0.19	0.078
			Controls	47.2	41.7	12.1				67.6	32.4			

Table 4

COMT Haplotype Association Results from UNPHASED for Stage 1 (N=188 cases, 188 controls; 196 males, 180 females) and Stage 2 (N=401 cases, 351 controls; 497 males, 255 females). Bolded text denotes p-values <0.05.

Stage	Markers	Haplotype	All			Men			Women		
			Frequency Cases	Frequency Controls	P-Value	Frequency Cases	Frequency Controls	P-Value	Frequency Cases	Frequency Controls	P-Value
1	4, 10	A-A	0.45	0.53	0.054 ^a	0.46	0.49	0.85 ^a	0.45	0.57	0.023^a
		A-G	0.066	0.065	0.077	0.087	0.089	0.56	0.043	0.040	0.046
		G-A	0.26	0.17	0.0069	0.23	0.19	0.77	0.29	0.15	0.66
		G-G	0.22	0.23	0.86	0.22	0.23	0.38	0.22	0.24	0.0024
								0.96			0.69
		A-A-A	0.42	0.48	0.29 ^a	0.43	0.47	0.89 ^a	0.41	0.49	0.16 ^a
		A-A-G	0.066	0.062	0.16	0.091	0.081	0.51	0.043	0.038	0.13
		A-G-A	0.13	0.091	0.52	0.089	0.091	0.98	0.18	0.088	0.64
		A-G-G	0.11	0.13	0.10	0.12	0.14	0.90	0.10	0.13	0.014
		G-A-A	0.037	0.045	0.57	0.022	0.024	0.59	0.053	0.069	0.59
2	4, 10	G-G-A	0.13	0.084	0.47	0.15	0.10	0.82	0.12	0.066	0.44
		G-G-G	0.10	0.099	0.042	0.10	0.080	0.20	0.10	0.12	0.078
					0.82	0.10		0.44	0.10	0.12	0.71
		A-A	0.46	0.46	0.066 ^a	0.47	0.46	0.58 ^a	0.43	0.47	0.029^a
		A-G	0.047	0.053	0.93	0.048	0.052	0.58	0.045	0.054	0.39
		G-A	0.27	0.21	0.39	0.25	0.23	0.67	0.29	0.18	0.37
		G-G	0.23	0.27	0.018	0.23	0.26	0.41	0.23	0.29	0.0033
					0.068			0.21			0.14
		A-A-A	0.42	0.43	0.12 ^a	0.43	0.43	0.23 ^a	0.40	0.44	0.12 ^a
		A-A-G	0.047	0.053	0.74	0.047	0.053	0.86	0.045	0.054	0.47
		A-G-A	0.13	0.098	0.36	0.13	0.11	0.59	0.13	0.079	0.40
		A-G-G	0.11	0.15	0.094	0.097	0.15	0.36	0.13	0.16	0.077
		G-A-A	0.036	0.029	0.020	0.042	0.022	0.026	0.028	0.045	0.28
		G-G-A	0.14	0.11	0.43	0.11	0.12	0.11	0.17	0.092	0.39
		G-G-G	0.12	0.12	0.099	0.13	0.12	0.86	0.10	0.13	0.015
					0.86			0.67			0.39

^a Global p-values for marker combination.

Table 5
Allele-based test statistics for *COMT* marker rs4680 or two-marker (rs4680, rs165599) G-A risk haplotype for entire female sample pooled across both stages (N=435).

Marker or Haplotype	q-values ^a			p-value	Allelic Odds Ratio ^b
	p ₀ = 0.95	p ₀ = 0.99	p ₀ = 0.999		
rs4680 (G allele)	0.59	0.88	0.98	0.0094	1.24
G-A haplotype	0.0012	0.0060	0.058	1.97e-5	1.95

^a False discovery rate for three values of prior probability of true effect p_0 .

^b Derived from stage 2 data only.

Table 6

Association analysis between individual phenotypes and marker rs4680 or two-marker (rs4680, rs165599) G-A risk haplotype (females only).

Phenotype	rs4680 (N_{cases}) ^a	G-A Haplotype (N_{cases}) ^a
Neuroticism	p=0.016 (415)	p=0.00001 (409)
Major Depression	p=0.024 (202)	p=0.0002 (202)
Generalized Anxiety Disorder	p=0.0026 (157)	p=0.00001 (156)
Panic Disorder	p=0.033 (67)	p=0.016 (66)
Agoraphobia	p=0.0013 (45)	p=0.00001 (44)
Social Phobia	p=0.0094 (43)	p=0.00007 (43)

^a N_{cases} represents the number of female cases available for analysis in the total sample for that phenotype (except for neuroticism, where the entire female sample was used). The numbers may differ slightly between the two columns due to variability in missing genotypes or haplotypes.