

# Cannabis, *COMT* and psychotic experiences

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## Background

A putative interaction between cannabis and variation at rs4680 within the catechol-methyl-transferase (*COMT*) gene on psychosis has been reported, but not adequately replicated.

## Aims

To examine whether the relative risk of developing psychosis following use of cannabis is dependent upon variation within *COMT*.

## Method

A longitudinal study of 2630 individuals from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort who completed questionnaire-based assessments for cannabis use at age 14 and incident psychotic experiences at age 16. Six SNPs within *COMT* were genotyped.

## Results

There was no evidence of an interaction under multiplicative models between cannabis use and *COMT* on the risk of

developing psychotic experiences in our primary analyses. In sensitivity analyses we observed highly variable evidence of interaction, whereby psychotomimetic effects of cannabis were greater in methionine homozygotes under some scenarios, but in valine homozygotes under others.

## Conclusions

Cannabis increases risk of psychosis irrespective of underlying *COMT* genotypes. These findings argue against the widely held belief that the relative risk of developing psychosis following use of cannabis is dependent upon variation within *COMT*. The public health message about the potential increase in risk of psychotic disorders following cannabis use should not be tempered by reports that this harm is subgroup specific in the absence of robust evidence of replication.

## Declaration of interest

S.Z. and M.J.O. have received contributions from pharmaceutical companies as honoraria for talks.

Cannabis use has been consistently associated with an increased risk of developing psychosis,<sup>1</sup> but risk factors for multifactorial complex diseases are of course rarely, if ever, necessary or sufficient to cause disease. Although it is very reasonable therefore to expect that the effects of using cannabis on risk of psychosis will vary across individuals in relation to the distribution of other risk factors, both genetic and non-genetic, the implications of findings from studies aimed specifically at examining gene–environment interactions have been challenged.<sup>2–4</sup>

The catechol-methyl-transferase (*COMT*) gene on chromosome region 22q11 encodes an enzyme that breaks down catecholamine neurotransmitters. A valine to methionine substitution at the single nucleotide polymorphism (SNP) rs4680 (Val<sup>158</sup>Met) within *COMT* results in reduced enzymatic activity and thus slower breakdown of dopamine, with evidence of a dose–response effect according to the number of methionine alleles present.<sup>5,6</sup> Although this polymorphism has been extensively studied as a putative risk factor for psychosis, results have been inconsistent.<sup>7,8</sup>

One study using the Dunedin cohort reported a putative interaction between cannabis and variation at rs4680 such that use of cannabis was associated with a 10-fold increase in risk of developing schizophreniform disorder at age 26 in individuals homozygous for the valine allele, whereas individuals homozygous for methionine appeared to be able to use cannabis without any increase in risk of developing psychosis.<sup>9</sup> Most studies have failed to support this finding,<sup>10–12</sup> or reported interactions but only in subgroups of individuals,<sup>13,14</sup> or with patterns of interaction that do not appear consistent with those in the Dunedin cohort.<sup>15</sup> There are therefore no studies to date that have replicated the relationship between cannabis and *COMT* on psychosis as originally described, yet this relationship remains a widely cited example of a gene–environment interaction in the psychiatric literature.

We aimed to examine further the relationship between cannabis, *COMT* and psychosis. Our measure of psychosis, age

at outcome and potential duration of exposure to cannabis differ from those in the Dunedin cohort as our cohort consists of younger individuals. We examine whether the relationship between cannabis use prior to age 15 and incidence of psychotic experiences at age 16 in a birth cohort study varies according to variation at rs4680 as well as at other loci within *COMT*.

## Method

### Sample

The Avon Longitudinal Study of Parents and Children (ALSPAC) cohort initially consisted of 14 062 children born to residents of the former Avon Health Authority area who had an expected date of delivery between 1 April 1991 and 31 December 1992 ([www.alspac.bris.ac.uk](http://www.alspac.bris.ac.uk)). The children, since 7.5 years of age, have attended annual assessment clinics where they participate in a range of face-to-face interviews, and have also completed postal questionnaires annually since they were age 6. There were 4263 children who participated in the psychosis-like symptoms questionnaires (PLIKS-Q)<sup>16</sup> at both 14 and 16 years of age (data restricted to 1 child per nuclear family). There were 530 individuals who responded 'Yes' to any psychosis questions at baseline (age 14 questionnaire), and these were omitted from the study. Genotype data (restricted to those of White ethnicity) was available on 2630 of these individuals (defined as the main sample). Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the local research ethics committees.

### Measures

#### Outcomes

The PLIKS-Q at age 16 consists of 11 questions covering hallucinations (visual and auditory); delusions (delusions of being

spied on, persecution, thoughts being read, reference, control, grandiose ability); and experiences of thought interference (thought broadcasting, insertion and withdrawal). Individuals were asked whether they had ever experienced these symptoms, and their frequency since their 15th birthday. The level of distress associated with each symptom was also measured. The questionnaire did not allow symptoms of cannabis intoxication to be identified separately from non-intoxication experiences. The 11 psychosis questions were based on the PLIKS semi-structured interview (PLIKSi) that has been used in this cohort at age 12.<sup>16</sup> Our primary outcome was a binary measure of a 'Yes definitely' response for any of the 11 psychotic experiences assessed.

#### Exposures

##### Cannabis

Data on cannabis use were obtained from self-report postal questionnaires completed at age 14. Information was obtained on whether an individual had ever used cannabis, the frequency of recent use and the total cumulative use over time. Our primary measure of exposure was cumulative use of cannabis reported at age 14 (none, <5, 5–20, 21–60, >60 occasions). Collapsing this measure into fewer categories by combining all those who used cannabis on >20 times into one group, or expanding this to separate use on 61–100 times from >100 times, made minimal difference to the results.

##### *COMT*

Our primary genetic exposure was variation at rs4680 (Val<sup>158</sup>Met), as examined in the original study.<sup>9</sup> We also examined interactions between cannabis use and a three-marker haplotype within *COMT* (SNPs rs6269–rs4818–rs4680) that has been shown to reflect *COMT* functional activity more accurately than variation at rs4680 alone (rs4633 that was part of this original haplotype was omitted as it was in perfect linkage disequilibrium with rs4680, with *D'* and *r*<sup>2</sup> of 1).<sup>17</sup>

As secondary analyses we also examined interactions between cannabis and three other SNPs typed within *COMT* (rs737865, rs2097603 and rs165599), selected on the basis that: they had been reported as being associated with psychosis in some studies; and they were not in high linkage disequilibrium (maximum *r*<sup>2</sup> = 0.32) with any of the other *COMT* SNPs typed.

Genotyping was performed by KBioscience (<http://www.kbioscience.co.uk>); SNPs were genotyped using the KASP SNP genotyping system. KASP is a competitive allele-specific polymerase chain reaction incorporating a fluorescent resonance energy transfer quencher cassette (for more information see <http://www.kbioscience.co.uk/reagents/KASP.html>). Genotyping was successful for over 97% of the sample across the six SNPs, with error rates of approximately 0.5%. None of the SNPs deviated significantly from Hardy–Weinberg equilibrium.

#### Confounders

A number of sociodemographic variables were considered as potential confounders in the interaction between cannabis and *COMT*: gender, parental social class (highest of both parents, based on occupation using the 1991 Office of Population, Censuses and Surveys classification),<sup>18</sup> parental welfare benefit, parental marital status, housing (own home, privately rented, council housing), urban/rural index at birth (urban/town, village/hamlet), childhood measures of victimisation (Bullying Questionnaire; age 8),<sup>19</sup> depression (Moods & Feeling Questionnaire; age 10),<sup>20</sup> emotional and behavioural problems (Strengths and Difficulties Questionnaire; age 10),<sup>21</sup> tobacco,

alcohol and other drug use (age 14), and family history of depression, schizophrenia or any mental health illness in biological parents or grandparents.

#### Sensitivity analyses

We undertook a number of sensitivity analyses to test out the robustness of our interaction results. These analyses examine to what extent variation in our conclusions occur when input parameters for the analyses are altered.

#### Sample

Data on psychotic experiences were also collected at ages 11 and 13 using questionnaires and at age 12 using a semi-structured interview. For our primary analyses we only used data from participants who responded to questionnaires at both 14 and 16 to maximise the size of our sample while maintaining a longitudinal study design. However, as sensitivity analyses we also examined: (a) the effect of omitting from the sample any individuals who endorsed any psychotic items as being definitely present from questionnaires completed at ages 11 or 13, or those rated as having suspected or definite psychotic experiences during a semi-structured interview at age 12 (restricted sample), and (b) the effect of relaxing our criteria to use the age 16 questionnaire data for our measure of cannabis exposure as well as our outcome, and irrespective of whether or not they had reported the presence of psychotic experiences at previous assessments. This was done to increase the prevalence of exposure to cannabis use, although at the cost of substituting the design from longitudinal to cross-sectional (cross-sectional sample).

#### Outcome

As sensitivity analyses we also examined (a) a narrower outcome of experiences rated as 'Yes definitely' that had either occurred at least once per month, or were rated as 'quite distressing' or 'very distressing', and (b) an outcome of only auditory or visual hallucinations that were endorsed as 'Yes definitely', as questionnaire assessments of these phenomena show higher validity than delusions when compared with interviews.<sup>16,22</sup>

#### Exposures

As sensitivity analyses we also examined associations using: (a) a binary measure of ever use of cannabis (no, yes) at age 14 (main sample and restricted sample) or age 16 (cross-sectional sample) and (b) average frequency of cannabis use (none, less than weekly, weekly or more frequent) at these ages.

#### Statistical analysis

Logistic regression was used to calculate odds ratios and 95% confidence intervals for associations between both (a) cannabis use and (b) *COMT* on psychotic experiences at age 16, both before and after adjustment for potential confounders. For all SNPs, we assumed an 'additive' genetic model, i.e. that the odds of our outcome increases proportionately with each additional minor allele copy. Haplotypes of rs6269–rs4818–rs4680 were derived using the software PLINK (version 1.06) for Windows.<sup>23</sup> Haplotype G–G–G has been reported as being associated with the greatest protein expression and highest *COMT* enzyme activity, whereas A–C–G was associated with lowest activity.<sup>17</sup> Individuals were coded according to the number of copies of the G–G–G haplotype that they had (high expression haplotype, coded as 0,1,2), and of the A–C–G haplotype (low expression haplotype, coded as 0,1,2). The haplotype probabilities were greater than

0.85 for over 95% of the sample (mean posterior probability 0.98 (s.d. = 0.05)).

Statistical interactions on a multiplicative scale between cannabis use and *COMT* genotypes or haplotypes on risk of psychotic experiences were investigated using likelihood ratio tests within logistic regression models. All analyses were conducted using Stata for Windows (version 11).

This study had >99% power to detect an interaction odds ratio of 3.5 as previously reported for cannabis and rs4680 on risk of schizophreniform disorder,<sup>9</sup> and >80% power to detect an interaction odds ratio of 2.0 as reported for self-reported hallucinatory experiences, at  $\alpha=0.05$ . Power calculations were conducted using QUANTO.

#### Missing data

The non-responders to PLIKS-Q were more likely to come from lower social class families, to have parents with lower education, to be male, and to be of minority ethnic status (all  $P<0.001$ ). Children not participating in the PLIKS questionnaire at age 16 were more likely to have used cannabis at age 14 (11% compared with 8%,  $P<0.001$ ) but did not differ by *COMT* genotypes ( $P$ -values 0.119 to 0.733).

## Results

Of the 2630 individuals in the main sample, 225 (8.6%) responded 'Yes, definitely' to any of the 11 psychosis questions at age 16, with 90 of these rating the frequency as occurring once or more per month or rating their experience as quite or very distressing.

### Cannabis and self-reported psychotic experiences

In the main sample, there were 168 individuals (6.5%) who reported having ever used cannabis at age 14. The odds of endorsing definite psychotic experiences at age 16 were 2.5-times greater in those who had used cannabis by age 14 compared with those who had never used cannabis by this age (95% CI 1.62–3.77,  $P<0.001$ ). There was evidence supportive of a dose–response relationship, with an odds ratio for linear trend across cumulative use categories of 1.60 (95% CI 1.28–1.99,  $P<0.001$ ).

### *COMT* and self-reported psychotic experiences

The distribution of self-reported psychotic experiences in relation to *COMT* genotypes is shown in Table 1 (rs4680) and online Table DS1. There was no evidence of association between

**Table 1** Number of individuals (%) endorsing definite psychotic experiences in relation to cumulative use of cannabis and *COMT* rs4680 genotype

	No symptoms <i>n</i>	Psychotic symptoms, <i>n</i> (%)
Cannabis use		
None	2209	194 (8)
<5 times	88	16 (15)
5–20 times	33	9 (21)
21–60 times	8	1 (11)
>60 times	6	3 (33)
<i>COMT</i> rs4680		
AA (met/met)	657	58 (8.1)
GA (val/met)	1158	119 (9.3)
GG (val/val)	532	47 (8.1)

psychotic experiences and any of the *COMT* SNPs or haplotypes examined.

### Cannabis × *COMT*

There was no evidence of an interaction under a multiplicative model between *COMT* rs4680 and cumulative use of cannabis on development of psychotic experiences (Table 2 and online Table DS2). Cumulative use of cannabis at age 14 was associated with an increased odds of psychotic experiences compared with non-use in individuals homozygous for the methionine allele (OR per one-category increase in cumulative use 1.56, 95% CI 1.05–2.31), and this association was very similar in heterozygotes (OR = 1.68, 95% CI 1.23–2.28) as well as valine homozygotes (OR = 1.47, 95% CI 0.85–2.26). Furthermore, there was no evidence that the strength of association between cannabis and psychosis differed according to the number of copies of the G–G–G (high activity) or A–C–G (low activity) rs6269–rs4818–rs4680 haplotypes (interaction  $P=0.373$  and  $P=0.499$  respectively), or across the other *COMT* SNPs examined (interaction  $P$ -values 0.304 to 0.574). None of the potential confounders adjusted for made any difference to the interaction results.

### *COMT* and cannabis use

The *COMT* genotype and cumulative use of cannabis at age 14 were not associated in the full sample with data available on both these measures ( $P=0.426$ ), although there was weak evidence that use was greater in methionine allele carriers compared with valine ones in the main sample used for analyses (OR per valine allele 0.86, 95% CI 0.73–1.01,  $P=0.063$ ).

### Sensitivity analyses

We also examined the main effects of cannabis and *COMT* rs4680, as well as their interaction in a number of sensitivity analyses (online Table DS3). We utilised different measures of exposure (ever use of cannabis, and average frequency of cannabis use), different measures of our outcome (psychotic experiences that occurred frequently or were distressing, and hallucinatory experience only), and different samples (a sample that omitted any individuals who endorsed definite experiences at any of the assessments prior to that at age 14 (omitted  $n=727$ ; restricted sample  $n=1844$ ), and a sample that used both exposure and outcome data from the age 16 assessment (cross-sectional sample  $n=3425$ ). For all of these 26 sensitivity analyses, main effects remained relatively stable for cumulative cannabis use, ever use of cannabis, frequency of cannabis use and *COMT* genotypes.

**Table 2** Interaction odds ratios (95% CI) between cumulative cannabis use and *COMT* genotypes on risk of self-reported psychotic experience

	<i>n</i>	Cannabis × <i>COMT</i> interaction	
		OR (95% CI)	<i>P</i>
rs4680	2567	1.00 (0.73–1.36)	0.981
rs737865	2525	0.87 (0.58–1.29)	0.488
rs2097603	2405	1.10 (0.79–1.51)	0.574
rs6269	2391	0.89 (0.64–1.24)	0.499
rs4818	2417	0.83 (0.60–1.18)	0.304
rs165599	2415	0.85 (0.60–1.21)	0.362
Haplotype (G–G–G) <sup>a</sup>	2357	0.86 (0.59–1.23)	0.373
Haplotype (A–C–G) <sup>a</sup>	2357	1.33 (0.58–3.05)	0.499

a. rs6269–rs4818–rs4680.

However, interaction effects showed greater variation. For example, the interaction odds ratio between cumulative use of cannabis and rs4680 in the cross-sectional sample indicated that risk of hallucinations from cannabis might be greater in valine homozygotes compared with methionine ones (interaction OR = 1.16,  $P = 0.039$ ); however, the interaction between cumulative use of cannabis and rs4680 in the restricted sample showed evidence of an interaction in the opposite direction, i.e. that risk of psychotic experiences from cannabis might be greater in methionine homozygotes compared with valine ones (interaction OR = 0.50,  $P = 0.034$ ).

## Discussion

In this study we found evidence of association between cannabis use and subsequent onset of self-reported psychotic experiences, consistent with other studies to date,<sup>1</sup> but no association between any of the *COMT* SNPs or haplotypes examined with this outcome. There was no evidence of statistical interaction under a multiplicative model between cannabis use and *COMT* rs4680, or between cannabis use and any of the other *COMT* SNPs or haplotypes examined on risk of developing psychotic experiences.

We are unable to make direct comparisons between our findings and those from the Dunedin cohort that examined schizophreniform disorder at age 26 as an outcome.<sup>9</sup> Nevertheless, our findings do not support a hypothesis that the relationship between cannabis and psychosis is moderated by variation at *COMT* rs4680, as originally reported. In the Dunedin study, the odds of developing schizophreniform disorder was approximately ten times greater in cannabis users compared with non-users in valine homozygotes, but was no different in methionine homozygotes. Similarly, the odds ratios for reporting hallucinations in relation to cannabis use were 5.3 and 1.2 in valine and methionine homozygotes respectively. In our study however, the association between cumulative cannabis use and onset of psychotic experiences was comparable across valine and methionine homozygotes (OR = 1.47 and OR = 1.56 respectively).

In our sensitivity analyses, main effects for cannabis use and for rs4680 remained relatively consistent, whereas the interaction effect showed much greater variability. Under some scenarios we observed evidence that the effect of cannabis on increasing psychotic experiences was greater in valine homozygotes than methionine ones, whereas under other scenarios we observed evidence to support a relationship in the opposite direction, i.e. that psychotomimetic effects of cannabis were greater in methionine compared with valine homozygotes.

### Possible explanations

It is possible that the reason we fail to find an interaction as previously reported is because of the different age group of our sample or the different measures of psychotic outcomes or cannabis use. Outcomes in our study were assessed at age 16 and cannabis use assessed at age 14, whereas in the Dunedin study these were assessed at ages 26 and 15–18 respectively. It is possible that the differential effect of cannabis use on psychosis according to genotype only becomes manifest during early adulthood, or is only present for more chronic use of cannabis.

Questionnaires almost certainly overrate psychotic experiences compared with semi-structured interviews, although they are not so dissimilar to structured interviews. The positive predictive value (PPV) of highly structured stem questions compared with semi-structured interview ratings in the ALSPAC cohort at age 12 was relatively high for auditory hallucinations (PPV = 70%) but much lower for most delusion items.<sup>16</sup> A similar finding of

reasonably good sensitivity and PPV for hallucinations was also reported in another study,<sup>22</sup> suggesting that although questionnaire-based assessments overestimate the true prevalence of psychotic experiences, self-reported hallucinations are likely to be a more valid measure of psychotic phenomena. Nevertheless, we found no consistent evidence to support an interaction between cannabis and *COMT* as originally reported when we examined incident self-reported hallucinations as our outcome in our sensitivity analyses.

Furthermore the Diagnostic Interview Schedule as used to assess psychotic experiences in the Dunedin cohort is a highly structured interview, and the frequency of psychotic experiences reported over a 1-year period was approximately double those endorsed from the questionnaire at age 16 in our study (26% compared with 13% for any psychotic experience; 13% compared with 6% for hallucinations). Variation according to age and location, as well as sample attrition in ALSPAC, might account for this difference, but nevertheless they suggest that overreporting of psychotic experiences within our study may be no more problematic than for studies that have utilised structured interviews.

The interaction reported in the Dunedin cohort was also observed when using informant reports of psychotic experiences, a measure that is also likely to be a less valid marker of psychotic experiences than an interview measure. Given all the above, and the fact that we observed a main effect of cannabis use on psychotic experiences in our study of a magnitude comparable with other studies that have used interview measures of psychosis, it seems rather unlikely that the lack of evidence of interaction between cannabis and *COMT* is due to misclassification of psychosis data.

We believe that a more likely explanation for our findings is that there is no substantive difference in the effect of cannabis use on psychotic outcomes between valine and methionine homozygotes at rs4680 under a multiplicative model. This is in keeping with other studies that have failed to support an interaction between *COMT* and cannabis as originally reported.<sup>11,12,14,24</sup> Furthermore, if an interaction effect size as originally reported were correct, we would also expect to observe a main effect for rs4680 without the need to study interactions given the frequency of cannabis use in the population. However, even in adequately powered meta-analyses the association between rs4680 and schizophrenia remains uncertain,<sup>7,8</sup> and a recent large-scale study also failed to provide support in favour of *COMT* as a susceptibility gene for schizophrenia.<sup>25</sup>

It is also possible that sample attrition may have biased our findings. In common with other large-scale longitudinal studies, missing data due to attrition and wave non-response was not insubstantial.<sup>26,27</sup> Differential attrition, whereby those who use drugs and have mental health problems are more likely to drop out, would be expected to lead to underestimation of the association between cannabis and psychosis. Genotype was not associated with missingness and it is unclear what effect attrition may have had on our results for interaction. It seems unlikely though that attrition bias would be an adequate explanation for the large variability in interaction results in the sensitivity analyses.

### Potential problems of studying gene–environment interaction

Spurious findings as a result of multiple testing are an important concern in studies of gene–environment interaction, and tests for interaction may also be more susceptible to the effects of subgroup testing and sample selection than those for main effects. For



example, although a cannabis  $\times$  *COMT* interaction was not observed in the primary analysis within one study,<sup>14</sup> there was evidence of an interaction when including another (three-way) interaction with a previous measure of psychotic phenomena (psychosis vulnerability). Furthermore, the interaction between cannabis and *COMT* reported in the original Dunedin cohort was observed only in participants who first used cannabis prior to age 18 years, with no evidence of interaction in those who first used after this age. Although it is possible that there are valid reasons why interaction effects are only observed in specific subgroups, it is also possible that such findings are spurious, and should probably be considered as such until robust evidence of replication exists.

It may also be that tests of interaction are more sensitive than main effects to changes in the way exposure or outcome measures are defined, and indeed in our sensitivity analyses the variation in results for main effects of cannabis and *COMT* did not appear to occur to the same extent as for the interaction between them. This increases concerns regarding the robustness of many putative interactions published to date, and emphasises the importance of strong and consistent replication before such reports are accepted as a true reflection of how exposures combine to alter disease risk.

Although power to reject the null hypothesis of a statistical test is in part determined by sample size, our findings indicate that compared with the study of main effects evidence of interaction may be much more susceptible to the effects of small numbers of events within subgroups, whereby the misclassification of only a small number of individuals may substantially alter findings or even reverse the direction of an interaction. The numbers of events in our study, when stratified by genotype, were very small. In our primary analyses for example, although there were 29 outcomes in those who had used cannabis, for the highest category of use (>60 occasions) there were only three psychosis outcomes in the methionine homozygotes and none in the valine ones. Yet by conducting a number of different analyses we observed evidence of two interactions that would lead us to diametrically opposite conclusions from the same set of data. Interaction results based on relatively few events are not uncommon in the gene-environment literature to date.

## Implications

Even if the relative risk of developing psychosis following use of cannabis does actually differ slightly across *COMT* genotypes, it is very questionable as to what extent such evidence would advance our understanding of aetiological mechanisms or inform potential strategies for prevention or intervention.<sup>4,28</sup> An interaction between cannabis and *COMT* as originally reported, where the effect of one exposure only occurs in the presence of another, could have potentially important implications in this regard. However, an interaction, if present, whereby cannabis increases the relative risk of psychosis in both valine and methionine homozygotes, but slightly more so in one homozygote group than another, would have a much more limited scope for potential benefits. This argument applies equally to potential interactions between cannabis and other polymorphisms that influence dopaminergic or other neurotransmitter pathways.

The public health message from our findings is somewhat different to that from the Dunedin cohort. Results from the Dunedin study found no evidence that risk of psychosis was increased by cannabis use in individuals who were methionine homozygotes.<sup>9</sup> It would be incorrect however to interpret this as meaning that methionine homozygotes can use cannabis with impunity in relation to risk of psychosis. Our results indicate that

cannabis use is likely to increase the risk of individuals developing psychotic experiences irrespective of underlying *COMT* genotypes, and argue against the widely held and commonly expressed belief that the relative risk of developing psychosis following use of cannabis is dependent upon variation within *COMT*.

The public health message about the potential increase in risk of psychotic disorders following cannabis use should not be tempered by reports that this harm is subgroup specific with respect to *COMT* in the absence of robust evidence of replication.

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