Author's response to reviews

Title: Antipsychotic medications and cognitive functioning in bipolar disorder: moderating effects of COMT Val108/158 Met genotype

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Title: Antipsychotic medications and cognitive functioning in bipolar disorder: moderating effects of COMTVal^{108/158}Met genotype

General reply:
We combined the Val/Met and Val/Val genotypes, on account of the fact that there was only a single individual with the Val/Val genotype using antipsychotic medication, yielding only one observation. Data were re-analysed.

A dummy variable was constructed for which the met/met genotype (n=12) represented value “0”, whilst value “1” represented presence of at least one val allele (i.e. combined val/met and val/val genotype; n=39), as described on page 9.

In general, changes in the text are highlighted in blue (in the ‘track-version’ of the manuscript), as shown on pages 2, 3, 4, 8, 9, 10, 11 and 15 (specified below).

Tables 1 and 2 were changed as per reanalysis of the data and the recommendations of the reviewers, as show on page 22/23.

Specific textual changes as requested by referees:

Page 1: ‘Marjan Drukker’ was added as third author (provided detailed power analyses).

Page 2: Section ‘Methods’ third line: ‘394’ changed into ‘379’. Section ‘Results’ third line ‘p=0.001’ changed into ‘p<0.001’. Section ‘Conclusions’ second line: ‘dose response’ left out; line 2 ‘negative’ added.

Page 3: Section ‘Background’ line 18: ‘and schizoaffective disorder’ added.


Section ‘Methods’ line four: ‘white’ added

Page 8: Section ‘Statistical analysis’ fifth line: ‘p<0.0042 (0.05/12)’ changed into ‘p<0.00625 (0.05/8)’ Bottomline: ‘including the demographic and cognitive data’ added.

Page 9: Line 4/5: ‘, symptoms (BPRS, HDRS, YMRS), and time as fixed factors’ added. Line 6/7: ‘value 1 for the Val/Met genotype and value 2 for the Val/Val genotype, with Val allele load as a continuous variable in a linear interaction model’ changed into: ‘and value 1 for the combination of the Val/Met and Val/Val condition. The Val/Met and Val/Val
conditions were combined because of the small number of observations in the Val/Val condition.

At the end of the ‘Method’ section a new subheading: ‘Power analysis’ was added.

‘Power analysis. ‘Power of the analysis was calculated by simulation from an example on the Stata website (http://www.stata.com/support/faqs/stat/power.html). Because these simulations cannot be performed in multilevel data, the unilevel equivalent of the n of the multilevel data set was calculated using the following formulae [40].

MF=1+(9-1)*0.6=5.8
uen=51*9/5.8=79

In which 9 is the number of assessments per person, 51 is the number of persons and 0.6 is the intra class correlation. The power of our sample size of n=79, alpha=0.00625 and a large effect size (0.8 sd) was 0.10’.

Page 10 : ‘Results’ section: first line: ‘symptom scores and’ added and ‘and allele frequencies’ left out. Second line: ‘three’ replaced by ‘two’. Third line: ; left out; ‘versus’ and ‘plus’ added , and ‘(p<0.0042)’ replaced by ‘(p<0.00625)’.

Line 3/4: ‘The Val/Met group had a higher GIT-IQ than the Met/Met group (effect size: 0.29; p= 0.026) and performed better on the working memory task than the Met/Met group (effect size:0.29; p=0.023)’ changed into: ‘The combined Val/Met plus Val/Val group performed better on the working memory task than the Met/Met group (beta: 1.15; p=0.033)’.

Line 5: ‘Twelve’ replaced by ‘Thirteen’.

Line 7: ‘Allele frequencies were 56 % for the Met allele and 45 % for the Val allele, respectively’ added.


Second line: ‘with higher Val allele load yielding larger negative effects’ left out.

‘Discussion’ section: first line: ‘moderating’ replaced by ‘modulating’ and ‘dose-response’ left out. Third line after’preliminary.’: ‘Replication of this underpowered, hypothesis-generating study would require a number of 193 patients in order to obtain a power of 0.8 (given the small number of patients using antipsychotics). Alternatively, increasing the number of patients using antipsychotics would increase power too’.Line 11-13: ‘This may explain our remarkable finding of an interaction between Val allele load and antipsychotics on delayed recall in a verbal memory test; a task requiring much cognitive effort’ was left out.

Reviewer 1: Sandra Dittmann

1. The authors do not describe with which statistical methods they have analysed the cognitive data as well as the demographic data. Even though they mention that there have been no differences between the three genetic groups concerning these data they should mention which analyses they have done. This is especially important, because of the small sample size.
The cognitive and demographic data were analysed using the STATA XTREG multilevel random regression routine with time as a random factor, with COMT<sup>Val<sub>108/158Met</sub></sup> (0 or 1) as dichotomous independent variable reflecting presence or not of the val allele, increasing the number of observations – as explained above. This has now been added to the text in the ‘statistical analysis’ section.

2. The authors have analysed the interaction of the COMT Val/Met polymorphism and antipsychotics using regression analyses. However some details have to be clarified. First of all, the authors have analysed the data on cognitive functioning over the period of two years. In the regression analyses they have included the time factor as a random effect. However, if subjects repeat cognitive tests several times the training effect is quite significant. Therefore the time effect in these analyses should not be a random but a fixed factor.

We apologize for not being entirely clear. Time as random factor refers to the fact that observations were clustered within individuals, compromising statistical independence of the data. This can be solved by making dummy variables of time, or by including time as a random factor in a multilevel random regression model (see Arts et al, 2011). The referee is quite right that in addition to this, the fixed effect of time is relevant for learning. This was addressed in a previous paper of ours (Arts et al, 2011). Thus, in order to quantify new learning, time was modelled as a linear variable in our earlier paper (Arts et al, 2011), yielding a rate of non-declarative learning of approximately 20% (the difference between the 50% cumulative rate of 2-year cognitive improvement versus the 30% cumulative rate of cognitive deterioration). In our opinion, however, this training effect is not relevant as either confounder, mediator or moderator for the interaction effects between COMT<sup>Val<sub>108/158Met</sub></sup> and use of antipsychotics, the topic of the article under review. Nevertheless, in order to accommodate the referee, we included this linear time variable in the regression model as fixed factor (see Table 2 on page 23).

3. The authors claim that they have used parallel tests to account for the training effect. But they have tested the patients every two months for a period of two years. Did they really use 12 different parallel tests to avoid those training effects?

Indeed we used 12 parallel versions of the verbal learning test in order to avoid test-retest-effects. We did not use parallel versions of the Flanker CPT, a measure of selective visual control of attention, or the Digit Span Backward, a measure of working memory, because of the hypothesized tiny training effects.

4. In addition, the authors should describe more in detail how they have classified the patients as far as the use of antipsychotics is concerned. The patients were examined over a time period of 2 years. The authors have written, that the use of antipsychotics was classified
with dummy variables of 0 and 1. However it remains unclear whether patients who were taking antipsychotics used them for the whole time period or if some started or stopped the use during the study period. If the patients started or stopped antipsychotic medication during the study period how was this accounted for? If every single visit of the patient was classified differently, the authors should have used a different statistical method (e.g. Marginal Structural Models).

We think there may be a misunderstanding. Antipsychotics were time-varying, i.e. could vary from observation to observation in the same person. Thus, twelve of the 51 bipolar patients used antipsychotic medications at any time during the 2-year period, contributing a total of 38 observations (page 10). Thus, our statistical model was the correct one to analyse these data.

5. Furthermore the authors should describe how the use of antipsychotics is distributed within the three genetic groups.

The distribution of the use of antipsychotics within the two groups (met/met versus val/met combined with val/val) is now included in Table 1 on page 22.

6. The authors do not describe the affective state the patients were in. Were the patients euthymic over the whole time period? Have the authors used rating scales for depressive and manic symptomatology? If so, the authors should state the mean scores of the rating scales in Table 1. If the patients had recurrences or subsyndromal symptomatology the authors should account for the symptomatology in the regression analyses.

We did use rating scales for psychopathology (BPRS; HDRS; YMRS), as described in our earlier paper (Arts et al, 2011). Psychopathology variables had significant, but small, negative effects on cognitive functioning, possibly due to the subclinical level of psychopathology in our study. Post-hoc analysis revealed no evidence for a nonlinear relationship between mood symptoms and cognition (Arts et al, 2011). The mean scores of BPRS, HDRS and YMRS are included in Table 1 on page 22. Most likely, patients had recurrences or subsyndromal symptomatology during the study period; the results of the regression analysis whilst taking into account symptomatology is shown in Table 2 on page 23.

7. Table 2 should be described in more detail. It is not clear whether table 2 only shows the interaction between antipsychotic medication and the genetic allele load. If this is the case, the two main effects (medication and genetic allele load) should also be presented. In addition psychopathology and time should also be accounted for as fixed factors and should be presented.
Table 2 was revised according to the comments above, as show on page 22. The associations with COMT<sup>Val<sub>108/158Met</sub></sup>, antipsychotics, and (stratified) interaction are described, with psychopathology and time as fixed factors.

**Reviewer 2: Jiang Li**

1. Genotyping section on Page 8, the author only reported SNP rs4680 but described a panel of 184 SNPs with Sequenom Massarray in detail. I am wonder if the rs4680, the only marker identified to be associated with the trait.

We only reported on SNP rs4680, which was selected a priori, because this is the only SNP that is consistently associated with antipsychotics in pharmacogenetic models, making it the only credible candidate. The selection of other SNP’s, interesting as they may be, would be a “fishing expedition”, because of lack of evidence. Furthermore, given the small sample size and number of observations, this study lacks power to look at more than one SNP. This has now been added to the method section for reasons of transparency (page 9: ‘Power analysis’).

2. In table 1, demographic information for ethnical group, antipsychotics used are missing. Allele freq is confusing and should be deleted and describe in the text. For Covariates listed in Table 1 is not sufficient. Antipsychotic drugs used are also the important confounding factor.

Table 1 on page 22 was revised according to the comments above. All subjects were white, as described on page 4. The use of antipsychotics is included in Table 1, and the allele frequency was deleted (described in the text on page 10).

3. The author use a verb, moderate or moderating throughout the text. The better word should be modulate.

The verbs moderate or moderating throughout the text are replaced by modulate or modulating on page 4 and 11.

4. Power analysis is missing since case # is limited without replications. See point1. A empirical power analysis was added in the manuscript on page 9.

5. Ancova test is necessary to evaluate if COMT rs4680 Val allele can predict the cognitive function outcome after treatment.
As we have shown that any main effect of COMT<sup>Val108/158Met</sup> on cognition after treatment is contingent on antipsychotic medication, the stratified effect is the valid measure to report. In **Table 2** on **page 23**, the stratified interaction effects are described, yielding the effect of 1 or 2 Val alleles (condition COMT<sup>Val108/158Met</sup>=1) on cognitive functioning in bipolar patients, treated with antipsychotic medication. The COMT<sup>Val108/158Met</sup> val allele is negatively associated with cognitive functioning for all measures, with verbal memory and the composite cognitive measure surviving Bonferroni correction.

6. In Result section on page 9, quote "there were no significant differences between ... after Bonferroni correction (p<0.0042). Please explain this.

We used the Bonferroni correction for multiple testing, yielding a corrected p-value of 0.00625 (0.05/8), as described on **page 8**. We combined the val/met and val/val subjects, yielding two groups of subjects analysed on 3 separate cognitive tasks, as well as on the composite cognitive measure (2x4=8). As shown in **Table 2** on **page 23**, highly significant findings, especially the interaction-effects on the composite cognitive measure, survived this Bonferroni correction, thus making a type I error less likely – although of course replication is necessary as mentioned in the discussion. Demographic and cognitive data in the two groups (met/met versus val/met combined with val/val), however, did not survive this correction (page 9)

7. In introduction and discussion section, the author mentioned about the different result between SNP rs4680 genotype and bipolar comparing to Schizophrenia. What about Schizoaffective disorders? This information should be added.

To the best of our knowledge, there is no literature on this topic. Some of the studies mentioned in our paper included schizoaffective patients, without specifying the results for this subgroup of subjects ((Weickert et al, 2004; Keefe et al, 2011). In general, these studies report positive effects of antipsychotics on cognitive functioning in these mixed groups of schizophrenia and schizoaffective disorder patients.

On **page3** we added ‘and schizoaffective disorder’