Author’s response to reviews

Title: Influences of polymorphic variants of DRD2 and SLC6A3 genes, and their combinations on smoking in Polish population

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Authors’ response to the Reviewers’ comments

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We appreciate the thoughtful and thorough comments offered by the Reviewers and the opportunity to revise our manuscript in accordance with their suggestions. Below is the Authors’ response to the Reviewers’ comments and information about manuscript revisions following these comments. For your convenience each comment is reproduced in italics and is followed by our response.

Reviewer 1 – Comments for the Authors

This manuscript reports the results of a genetic association study of the DRD2 Taq1A and SLC6A3 VNTR polymorphisms and smoking status, and a range of smoking-related phenotypes such as age at initiation and heaviness of smoking. The manuscript is well-written and concise. However, the small sample size is a considerable concern. The proliferation of under-powered genetic association studies has led to concerns that the literature may contain more false positives than true positives, and that this hampers our attempts to further our understanding of the neurobiology and genetic architecture of complex phenotypes such as tobacco dependence. The authors are correct that simple categorical phenotypes such as smoking status may not do justice to the complexity of smoking behaviour, but this advantage (of testing multiple phenotypes) is offset in this study by very low statistical power and the use of uncorrected alpha levels for statistical significance.
These problems are further compounded by the investigation of multiple genetic variants (and their interactions). This, in conjunction with the multiple phenotypes tested, raises the multiple statistical testing burden considerably. It is also not clear whether any further testing of unreported genetic variants took place.

The study is technically sound (in terms of phenotype definition, genotyping, etc.), and I am sympathetic to the need to publish the results of all genetic association studies to minimise the risk of publication bias, but this need to be weighed against the risk associated with further flooding the literature with false positives.

MAJOR ESSENTIAL REVISIONS
A brief report, clearly describing the number of genetic variants (and their interactions) and the number of phenotypes examined, using a corrected alpha level for the corresponding number of statistical tests, would be more helpful, even if this means that the reported results are described as non-significant.

AUTHORS’ RESPONSE: We agree that the small study size calls for its cautious interpretation. According to the Reviewer, it is insufficient to detect small genetic effects of the DRD2 Taq1A and SLC6A3 VNTR polymorphisms on smoking status, which are likely for single loci and complex behavioural phenotypes. Although several studies of similar size have been published in the past, there is growing consensus that sample sizes in 1000s will be necessary to provide sufficient statistical power. Nevertheless, we stand by our opinion that in spite of sample size limitation the study adds value to the current knowledge, whereas its limitations are addressed in the Discussion section. Of note, the Reviewer 2 has not raised the issue of the sample size. As presented in the study, we found some associations between studied polymorphisms and smoking-related phenotypes. The Reviewer expressed his concern that these results might be false positive due to using of uncorrected alpha level for the corresponding number of statistical tests. Indeed, we did not use Bonferroni correction, as the need to correct for multiple testing for statistical calculations remains controversial, and was discouraged by some authors (e.g., Perneger T. V. What’s wrong with Bonferroni adjustments. BMJ, 1998, 316: 1236-1238). However, taking into consideration the Reviewer’s concern in the revised manuscript we have corrected alpha level for multiple testing and reported significance thresholds after Bonferroni correction (Table 2 and 3). This method did not considerable change our results, and the main correlations remained significant. Given that smoking is a polygenic trait, we assumed that combinations of two polymorphisms, rather than one polymorphism, may be most strongly associated with smoking status, as well as smoking related-phenotypes, therefore we did not correct alpha-level for multiple testing for genotype combinations. Taking into account that DRD2 and DAT
genes have direct functions in the dopaminergic pathway, we consider our findings relatively sound and robust. In our opinion studies of sample size in 100s may help in generating hypotheses on the neurobiology and genetic architecture of complex phenotypes such as those related to tobacco dependence.

We do hope that our study may encourage other researchers to replicate our results on a larger sample.

Reviewer 2 – Comments for the Authors

The present study provided additional information on the associations between smoking habits and genetic polymorphisms of DRD2 and SLC6A3 among a Polish population. The description is sufficient and precise, but the p-values of Hardy-Weinberg will be helpful for the readers to realize the degree of deviation from the expected genotype frequencies

AUTHORS’ RESPONSE: P-values of Hardy-Weinberg have been added.