Reviewer’s report

Title: Lack of association between serotonin transporter gene polymorphism 5-HTTLPR and smoking among Polish population: a case-control study.

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Reviewer: Jerzy Samochowiec

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Revision of Manuscript.: Lack of association between serotonin transporter gene polymorphism 5-HTTLPR and smoking among Polish population: a case-control study” by A. Sieminska et al.

The Authors of the Manuscript investigated the associations between 5-HTTLPR gene polymorphism and vulnerability to tobacco smoking. They examined 149 ever smokers (66 females; mean age 53.0 years) and 158 gender and ethnically matched never smoking controls (79 females; mean age 45.0 years) to evaluate the association of this polymorphism with smoking status. They have analyzed the age of starting regular smoking, the number of cigarettes smoked daily, pack-years, FTND score, duration of smoking, and the mean length of the longest abstinence on quitting.

The authors found no significant differences in the rates of S allele carriers in ever smokers and never smokers, and no relationship was observed between any quantitative measures of smoking and the polymorphism.

They performed a multivariate analysis, which demonstrated a significant association between the older age (OR=3.99), alcohol dependence (OR=10.13), and the higher ratio of smokers among first-degree relatives (OR=4.03) and smoking.

The authors concluded that although a higher risk of smoking among subjects reporting greater family aggregation of smokers supports a genetic basis for this behaviour, the role of 5-HTT gene may not be significant. Probably, other factors, including non-genetic influences, more strongly affect the susceptibility to smoking than 5-HTTLPR.

My comments:

1. Unfortunately, the Authors did not use SCID, SCAN or even MINI to establish the diagnosis and comorbidity. The authors have also given a rather poor characterization of their investigated group.

In such a type of studies the evaluated population should be chosen appropriately according to the statistical population of a given region (according to the education level, age and sex ).

The subjects should, at least, have been examined using such questionnaires as SCID, SCID II or SCAN or even Prime MD (Primary Care Evaluation of Mental
Disorders) which would have excluded psychiatric disorders of axis I.

The authors of the Manuscript speculate that there is growing evidence indicating that smoking behaviour and ability to quit are influenced by personality traits, including neuroticism, and psychiatric disorders or alcohol dependence. There are data also suggesting that smoking behaviour is influenced by an interaction between neuroticism and 5-HTTLPR genotype.

As an example of importance of careful inclusion of probands in such studies the authors should have quoted results, obtained by other Polish research groups investigating patients from the north of Poland:

a/ While examining controls (who were 6th year medical students) the 5-HTT gene promoter polymorphism, heterozygous individuals (l/s) and individuals with 44-bp deletion (s/s) scored significantly lower in the HA1 subdimension (anticipatory worry and pessimism vs. uninhibited optimism). Neuropsychobiology. 2001;43(4):248-53.

b/ A few years later, while examining controls, this time selected (without psychiatric disorders) and recruited to represent a cross-section of the population of Szczecin (Poland) in terms of sex, age and education –no associations between 5-HTT-LPR and the TCI harm avoidance dimensions and between 5-HTT-LPR and the NEO-FFI neuroticism dimension were found. Neuropsychobiology. 2004;50(2):174-81.

2. The authors are aware of the limitations of their study, and explain that the main purpose of their study was to assess the relationship between 5-HTTLPR and smoking, and psychiatric disorders in general, as well as alcohol dependence, served only as covariates in their analyses.

3. The paper is well written, the conclusions are well justified but the review of the state-of-the-art literature is inadequate (i.e. mentioned earlier in point 1).

4. Although the authors proposed a plausible hypothesis, some methodological problems are still to be clarified:

23 subjects (7.5%; 15 females) reported a current or prior treatment because of psychiatric disorders, including depression or anxiety-related disorders (20 subjects), and schizophrenia (3 subjects). The cited medications were in agreement with self-reported psychiatric diagnoses and included inhibitors of selective serotonine transporter (15 subjects), tricyclic antidepressants (4 subjects), neuroleptics (3 subjects), and anxiolytics (1 subject). Seventeen subjects (5.5%; 3 females) in the study group mentioned alcohol dependence in the self-report.

That makes this population rather heterogenous.

Maybe the Authors should calculate the results in patients with no comorbidity and show it in separate table again. It could at least help to interpret the influence of 5-HTTLPR directly on more homogenous groups of smokers vs non-smokers.

In conclusion:
1. Is the question posed by the authors well defined? Yes
2. Are the methods appropriate and well described? Yes, although I have stated above my doubts towards the methodology
3. Are the data sound? Yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes
5. Are the discussion and conclusions well balanced and adequately supported by the data? The Manuscript needs a few more literature positions to be discussed.
6. Are limitations of the work clearly stated? Yes
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes
8. Do the title and abstract accurately convey what has been found? Yes
9. Is the writing acceptable? Yes

There are some major revisions to be made owing to a lack of calculations of 5-HTTLPR in homogenous subgroup of smokers without co morbidity. Some literature data should be added.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests' below