Reviewer's report

Title: Mutation screening of melatonin-related genes in patients with autism spectrum disorders

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Reviewer: Dawn Wimpory

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Essentially the manuscript reports another case of ASDs carrying the IVS5+2T>C variant in ASMT. Additionally, two new SNPs are reported in the promoter region of the ASMT gene. In GPR50 a known coding variant is highlighted which is previously reported as rs62620754 in the public databases. In MTNR1A a novel SNP in the 5'UTR is found, while in MTNR1B, two coding variants are highlighted: one novel (V124I) and one previously reported, K243R (rs61747139). A novel variation (c-39GC>AA) is also reported in the 5'UTR of this gene in the ASD sample.

Major Compulsory Revisions (5 points)

1) The text does not clearly express which SNPs are novel variants found by this study and which have already been reported as SNPs in the publicly accessible databases. As the variants c1478 G>A (rs62620754) in GPR50 and c728 A>G in MNTR1B (rs61747139) are variations already in the dbSNP database, these SNPs should be reported as such. Even though the population diversity of these database SNPs is currently undefined, calling these SNPs rare variants is confusing and also conflicts with the results in Table 1. Regarding the statement that begins the Results section:- “Six rare variants and two previously unreported polymorphisms in the investigated genes were found during the screen (Table 1).” This could be misleading. This statement should be corrected and table 1 amended (add rs codes for example) to differentiate novel SNPs from known SNPs.

2) Focusing the results on the novel findings would put more emphasis on the bioinformatics analysis, which should be enhanced. For example, three alternative transcripts for ASMT are known. These are not considered in the report. Where are the novel 5’ SNPs in relation to the ASMT alternative transcripts? It appears that the position of the novel SNP c-38C>T and that of the ASMT-expression-altering SNP rs5989681 is identical (one nucleotide 5’) relative to the start of the ASMT-001 and ASMT-002 transcript respectively, this is interesting. Indicate the extent of the 5'UTRs of the alternative ASMT transcripts in figure 2 and use rs SNP codes and perhaps genome co ordinates to help in the presentation and discussion of this data in the text and figures.

3) For the analysis of the novel variant c370G>A in MNTR1B where peptide sequence conservation is depicted, the valine residue also appears to be
conserved in GPR50. This is not considered in the manuscript. This should be considered and discussed as this may alter the impact of this finding either way. The alignments should be shaded and discussed in the context of protein families/clans and functional domains.

4) Re: Line 3/ page 6/, sentence starting “Pateints from both populations…” Did all the patients from each population get both DSM-IV and ADI-R assessment? If not give the % for each assessment in each group. If all the patients received both assessments insert “All” at the beginning of the sentence.

5) Regarding the use of terms, we consider the unaffected sample to be more of a comparison group than a control group. Indeed, the authors state (page 6 line 10) “The control group in this study served only to investigate if mutations identified in patients were present also in the general population”. This point however is evident from the SNP and human genome sequence databases. The comparison group merely gives us an indication that the specific population surveyed is in keeping with the human genome database information regarding common SNPs. It does not properly act as a control, as the authors rightly point out. The terms “control” and “control group” should thus be replaced with an alternative, more appropriate term in a number of places in the manuscript.

Minor Essential Revisions (6 points)

1) Abstract/ Page 3/ Conclusions/ line 2: Moreover, our results suggest that also other melatonin… should read…Moreover, our results also suggest that other melatonin…

2) Background/ Page 4/ …. Break this into paragraphs at line 14 and 19 say.

3) Methods/ Page 5/ line 10/ Sachsska childrens hospital…presumably should be Sachsska Children’s Hospital

4) Methods/ Page 6/ line 3/ … Spelling… Pateints should be Patients.

5) The grammar of the first sentence of the second paragraph of the Discussion requires correction and in the third sentence of the Discussion we read, “Moreover the most interesting of the identified variants were identified in patients…etc.” Which variants? Please specify and say why they are the most interesting.

6) In addition to ref. 21 line 8 page 10, cite Hu VW et al., (2009) “Gene expression profiling differentiates autism case-controls and phenotypic variants of autism spectrum disorders: evidence for circadian rhythm dysfunction in severe autism”.

Discretionary Revisions

Consider using additional bioinformatics tools to investigate the novel SNPs more thoroughly rather than the promoter regions in general. For example, an alternative scan tool might pick up a site that is altered by the c-39 G,C >A,A variation in MNTR1B. It is a pity that the work is unsupported by any association tests on the novel SNPs or the effect of the novel promoter region SNPs on gene
expression but overall this manuscript offers some new data that could inform future expression studies and population genetic analysis of melatonin related genes in autism.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'we declare that we have no competing interests'