Reviewer’s report

Title: C4B null alleles are not associated with genetic polymorphisms in the adjacent gene CYP21A2 in autism

Version: 1 Date: 21 August 2007

Reviewer: Chack Yung C Yu

Reviewer’s report:

General

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

General Critiques/Comments:

Autism affects male subjects four times more frequently than females. The authors’ group has made original observations on the increased frequencies of heterozygous or homozygous deficiency of complement C4B in autistic-spectrum patients of European origin. Located 3-kb downstream of the complement C4B gene is the CYP21A2 or CYP21B gene, which encodes the cytochrome P450 21-hydroxylase that is essential for the biosynthesis and metabolism of cortisols and mineralocorticoids. A complete deficiency or malfunction of the 21-hydroxylase can lead to excessive accumulation of male sex hormones. Gene copy number variation of complement C4 is always concurrent with its three neighboring genes including CYP21 in the RCCX modules. It is therefore a natural and relevant extension for the authors to ask whether deficiencies of C4B in the autistic subjects are associated with deleterious mutations of CYP21A2, leading to a higher frequency of male autistic subjects. Although the answer appears to be negative, the results can help with our understanding of the genetic basis of autism.

The following minor revisions are suggested to improve the clarity of the manuscript:

1. Results of this study suggested that autistic subjects with homozygous or heterozygous deficiency of C4B have very low frequency or do not have the common mutations in the neighboring CYP21A2 genes. However, it is not clear whether are any promoter polymorphism or epigenetic variation in CYP21A2 that might affect the protein expression levels or the enzymatic activities of CYP21. In addition, the sizes of study populations are relatively small (n = 80 for patients and 60 for controls). There may not have enough statistical power to make a broad conclusion for less frequent mutations of CYP21A2 in autism. The authors may want to slightly temper their negative conclusion of CYP21A2 mutations in autism in general.
2. Please provide greater technical details to explain how specific PCR amplifications of CYP21A2 (but not CYP21P) genes were achieved in the mutation detection assays. Please comment on the frequencies of trimodular RCCX in the patient and control populations, if possible. When there are more than two copies of CYP21A2 genes in a subject, would the mutation detection assays be sensitive enough to detect changes in only one of the CYP21A2 genes?

3. Please correct a typographical error on page 2, second paragraph, the first word of the sixth line should be RP2 instead of RP1

4. In the legend of figure 1, it is stated that monomodular RCCX with C4B deficiency appeared in 19 chromosomes and bimodular RCCX with C4A-C4A genes in 21 chromosomes of the 80 autistic subjects. The authors may want to compare the frequencies of these haplotypes with healthy subjects and determine if there were significant differences.

**What next?:** Accept after minor essential revisions

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

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

'I declare that I have no competing interests'