Author's response to reviews

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

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Author's response to reviews: see over
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Dear Editor(s) BMC Medical Genetics,

Thank you for the thoughtful review of our manuscript (MS: 3290742791541646) entitled, “C4B null alleles are not associated with genetic polymorphisms in the adjacent gene CYP21A2 in autism.” Each of the referee’s requests is addressed below.

Referee 1
1. The reviewer suggested slightly tempering the negative conclusion because it is not clear whether CYP21A2 promoter polymorphisms or epigenetic variations that were not determined might affect protein expression levels or the enzymatic activities of CYP21. As well, there was a concern about statistical power for detection of less frequent mutations.

Therefore, at the end of the conclusion we added the following statement, including reference 28.

“However, a role for CYP21A2 in autism cannot be ruled out as other factors affecting CYP21A2 gene expression such as promoter polymorphisms or epigenetic variation were not studied and may be relevant [28]. As well, a weak association may be beyond the statistical power of the present study to detect.”

2. Greater technical detail was asked for to explain how specific PCR amplifications of CYP21A2 (but not CYP21P) genes were achieved in the mutation detection assay.

Therefore, the following explanation was added to the polymerase chain reaction subsection of the methods section.

“Each reaction contained a primer specific for either the common or rare genetic variant in conjunction with a primer that amplified only the CYP21A2 gene and not the pseudogene (Table 1).”

The referee asked for comment on the frequencies of trimodular RCCX in the patient and control populations, if possible.

Therefore the following sentence was added to the end of the legend for figure 1.

“No C4B null alleles or CYP21A2 mutations were detected in subjects (2 autistic, 4 control) with trimodular RCCX modules, determined by protein immunofixation [10].”

The referee asked if the PCR assays were sensitive enough to detect changes in only one CYP21A2 gene if there were more than two copies of the gene.
In response the following sentence was added as the second sentence in the polymerase chain reaction subsection of the methods section.

“This method is as accurate as the dot blot procedure [17]; therefore, it is sensitive enough to detect a mutation in only one CYP21A2 gene if more than two copies of the gene are present.”

3. “RP1” was changed to “RP2” on page 2, second paragraph, the first word of the sixth line.

4. Frequency of the monomodular RCCX with C4B and bimodular RCCX with C4A-C4A were presented in the legend of figure 1. A comparison of the frequency of these haplotypes compared to the healthy subjects was requested.

Therefore, this comparison was completed and the following statement was added near the end of the legend for figure 1.

“In the control subjects with C4B null alleles 10 chromosomes were monomodular and 1 was bimodular. Bimodular C4B null alleles were significantly more frequent in autistic subjects compared to controls (P=0.0001).”

Referee 2
Referee 2 requested that a reference in the legend of figure 1 be changed to “[7]” instead of “(Blanchong et al 2000).”
This was done.

Also, one of our authors suggested a correction in the second sentence of the background section which read, “Approximately 1 in 152 children receive a diagnosis of an autism spectrum disorder [1].” The correct term here is prevalence.

Therefore, the sentence was changed to read; “The current prevalence for the disorder is approximately 1 in 152 children [1].”

Best Regards,

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