Author's response to reviews

Title: Association study of SHANK3 gene polymorphisms with autism in Chinese Han population

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Author's response to reviews: see over
Dear Editors,

We thank you and reviewers for careful review our manuscript entitled “Association study of SHANK3 gene polymorphisms with autism in Chinese Han population” (Manuscript ID: 1931124942248626). And we appreciate the reviewer’s comments and suggestion. We have answered every question of the reviewers and revised our manuscript accordingly. We believe that our manuscript in its current form is suitable for publication at BioMed Central.

Thank you for your consideration.

Sincerely yours,

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First, we would like to express our sincere appreciation to these two reviewers for their valuable comments and excellent suggestions which greatly improved our manuscript. The original comments of the reviewers are shown in italics and followed by our responses.

Answers to reviewers

**Reviewer:** Mariko Y Momoi

**Reviewer's report:**
The authors presented their negative data on the contribution of SHANK3 gene to autism after analyzing SHANK3 sequences of Chinese patients and controls, and discussed that SHANK3 may not be a major candidate gene for autism. They did not detect significant link between SHANK3 SNPs and affected condition and did not detect mutation either that was specific to the patients. Their study was precisely done and their data were properly analyzed.

However, we know that the mutation of a certain candidate gene is detected among less than 1% of the affected, and the negative data about the specific SNPs or mutations in the study scale presented by them does not add any scientific information we need. I regret that the data they reported does not have sufficient importance in the area of autism.

**Reply:**
Recent research reported that genes which may be involved in the process of synaptogenesis are associated with autism. So researchers hypothesized that autism’s cause may reside in abnormalities at synapses. Shank3 acts as a binding partner for neuroligins(NLGN) and the “neuroligin autism pathway” of autism has been postulated (Meyer, et al. 2004). Moreover, it plays a role in synaptogenesis. In this study we explore whether the SHANK3 gene was associated with autism in the Chinese Han population. Although several de novo mutations in SHANK3 have been identified, the linkage and association evidence was insufficient. To determine whether SHANK3 is a major autism susceptibility gene, we performed a family-based association study to analyze the transmission of allele in 305 trios. We choose 7 SNPs in SHANK3 gene. Among these, 2 SNPs has no polymorphism in our samples. So at last five SNPs (MAF>5%) were used as genetic markers for the association study. We also used direct DNA sequencing for the screen of the rare de novo mutations reported by Durand et al., Moessner et al. and Gauthier et al..

We didn’t detect the association of SHANK3 gene and autism. Nor did we find the previous reported mutations in our samples. Our study indicates that SHANK3 is unlikely to be a major autism-associated gene in the Chinese Han population. As
detailed in the Discussion section, these results may be explained by differences in
c polymorphisms between the Chinese Han population and other ethnicities, and the
fact that our samples only include infantile autism trios and not the wider range of
ASD. Nonetheless, since we did not find any evidence for association between
SHANK3 SNPs and autism, we raise the possibility that SHANK3 may not be a
major susceptibility gene for autism, at least in the Chinese Han population. We
believe that this is an important result in the autism field.
Reviewer: Roberto Giorda

Reviewer's report:

1. Major Compulsory Revisions

1.1 The authors have analyzed several SNPs in the SHANK3 gene for association with autism in 305 Chinese Han families, and found no association. They refer to additional experiments of high-throughput genotyping on 240 trios showing no copy-number variations in and around the SHANK3 gene. In my opinion, these data should be incorporated in the present report, as they complete the genetic analysis of the region.

Reply:

We performed the genome-wide association study using Affimetrix SNP 5.0 chip. The results of SNPs association study and CNV analysis have been submitted in another article, which is a major piece of work completed by another member of the laboratory. Since this other piece of work has not yet been accepted for publication, in the present study we can only mention this information as unpublished data and could not incorporate the complete data in the present report.

1.2 I would also recommend testing whether the Affimetrix 5.0 chip is able to detect the small recurrent deletion described by Bonaglia et al, 2006, Durand et al, 2007, and others.

Reply:

Thank you for your good suggestion. We carefully tested whether the Affymetrix SNP 5.0 chip include the CNV probe sets in SHANK3 gene. We checked the location of the CNV probes and found that 16 CNV probes were located in SHANK3 gene and its flanking region. Among these CNV probes, there are some located at the region reported previously. It is indicated that the Affymetrix SNP 5.0 chip could detected the small recurrent deletion described by other previous researches.

The location of these 16 CNV probes is shown below.

<table>
<thead>
<tr>
<th>Probe Set ID</th>
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</tr>
</tbody>
</table>
Revisions:
Page 8, 2\textsuperscript{nd} paragraph was revised as: “The \textit{SHANK3} gene spans about 60kb. In Affimatrix SNP 5.0 chip of 240 trios from this sample, it included 16 CNV probes covering \textit{SHANK3} gene and its flanking region. The smallest distance between two probes was less than 500bp. In these probes, one CNV probe was located at intron8 which was reported as a breakpoint of a de novo deletion\cite{17}, and a few CNV probes were quite close to exon21 which was reported as a breakpoint of a de novo translocation in 22q13 deletion syndrome\cite{7}. However, we didn’t find any genomic imbalance in all these 240 trios or control (unpublished data).”

2. Minor Essential Revisions

2.1 The authors point out in their Discussion that the association between \textit{SHANK3} dosage and autism is controversial. I would suggest that the 22q13 deletion syndrome has a clinical phenotype overlapping in part the ASD phenotype, and sometimes subjects with 22q13 deletion are included in ASD cohorts. Choosing autistic patients with stricter criteria may help to exclude 22q13 deletion patients from the study group. On the other hand, \textit{SHANK3} haploinsufficiency has been definitely associated with the major clinical signs of 22q13 deletion syndrome in over 300 subjects while the cases described by Wilson and colleagues are exceedingly rare. The authors should rewrite this paragraph accordingly.

Reply: We appreciate the reviewer’s excellent suggestion. As the reviewer mentioned, 22q13 deletion syndrome has a clinical phenotype overlapping in part the ASD phenotype. And the location of \textit{SHANK3} is special. It is really critical for the researchers to set up stricter criteria of their samples.

Revisions: Page 10, line 6: “We didn’t detect any genomic imbalance of \textit{SHANK3} and its flanking regions in our sample by Affimetrix 5.0 chip either.”

Page 11, line 5: “The location of \textit{SHANK3} is at 22q13.3, which is a critical region for 22q13 deletion syndrome that also has autistic behavior. There are strong evidence that haploinsufficiency of \textit{SHANK3} plays a major role in 22q13 deletion syndrome\cite{30}. \textit{SHANK3} is very likely to be involved in the pathogenesis of some mutual phenotypes of ASD and 22q13 deletion syndrome, such as delay of expressive speech. Furthermore, 22q13 deletion syndrome has a clinical phenotype overlapping in part the ASD phenotype. So subjects with 22q13 deletion may be included in ASD samples. It is really critical for the researches on autism to exclude the 22q13 deletion syndrome. We didn’t detect any genomic imbalance of 22q13 in our samples using Affimetrix SNP 5.0 chip. Moreover, although the disruption of \textit{SHANK3} seems to be associated with 22q13 deletion syndrome, there is also contrary evidence that the haploinsufficiency for 22q13 genes other than \textit{SHANK3} have major effects\cite{31}. The present study indicates that \textit{SHANK3} may not be a
critical gene for the etiology of infantile autism in Chinese Han population. As autism is a heterogeneous disease, the rare mutations of \textit{SHANK3} gene seem to explain the etiology of only a small proportion of cases with autism. Sykes et al. reported recently that they didn’t find any CNV or SNP association of \textit{SHANK3} within their ASD sample, although they didn’t sequence the gene\cite{32}. Their suggestion that \textit{SHANK3} deletions may be limited to a portion of autism was coincident with ours.”

3. Discretionary Revisions

Although the style of the paper is generally clear and readable, there are a few unusual sentences (e.g. Page 3, last line) and several grammatical errors.

Revisions:

Page 2, background, last sentence: “Rare mutations and copy number variation (CNV) evidence suggested \textit{SHANK3} as a strong candidate gene for the pathogenesis of autism.”

Page 3, last line: “Synapses are the physical sites through which neurons in the brain connect with each other into an integrated circuit. In 2003, the alterations in synaptic function was first proposed to be a possible cause of autism\cite{11}.”

Page 6, line 4: “Mutation screen for the rare de novo mutataion reported previously was performed by sequencing exon8 (for A962G\cite{18}), exon21 (for G insertion\cite{17}) and donor splice site G deletion of intron 19\cite{19} in all the 305 probands with autism.”

Page 8, line 10: “To determine whether any specific haplotype would confer a higher risk for autism, we tested all specific and globe haplotype composed of these SNPs. Two haplotypes displayed weak association with autism.”

Page 10, line 11: “We didn’t detect any genomic imbalance of \textit{SHANK3} and its flanking region in our sample using Affimetrix 5.0 chip either.”