Author's response to reviews

Title: An investigation of ribosomal protein L10 gene in autism spectrum disorders

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

We want to thank the editors and the reviewers for their critical reading of our manuscript. We answered all questions and modified the text accordingly. Since the submission of our report, we could analyze 91 of the 141 patients with ASD using the Illumina 1M SNP array. The whole genome analysis is not finished yet. Nevertheless, we already screened for CNVs within or close to RPL10 and found no genomic imbalance in the patients. We included this information in the text as unpublished results. These new genomic data do not prompt us to pursue on this gene and give support that RPL10 is not altered in our sample of ASD patients. Please find below all the modifications requested by the reviewers.

Sincerely yours,

Pr. Thomas Bourgeron

Reviewer 1: Sabine M Klauck

Authors only mention the sequencing of all exons and flanking sequences of RPL10 and stated that they have used the primers provided in our report (Klauck et al. 2006). This included also sequencing of the U70 snoRNA. If this has been performed in the patient sample as well it should be mentioned in the Methods and Results section for completion.

The sequencing of the U70 snoRNA was not done in our sample.

Methods, page 4, line 4 and 5: References for the ADI-R and ASDI should be given.

Correction:

Page 4, line 2: “The patients were evaluated by experienced psychiatrists or child neurologists and assessed with the Autism Diagnostic Interview-Revised (ADI-R) or the Asperger Syndrome Diagnostic Interview (ASDI),” is changed to “The patients were evaluated by experienced psychiatrists or child neurologists and assessed with the Autism Diagnostic Interview-Revised (ADI-R) [9] or the Asperger Syndrome Diagnostic Interview (ASDI) [10].”

Reviewer 2: Astrid Vicente

As the authors correctly state, there are important limitations in this study, namely the population sample size, which is too small and therefore has very limited power to detect rare mutations. While for complex disorders such as autism it is very important to report negative results, namely the non-replication of previous findings of mutations or associations, it is also fundamental that the follow up studies are appropriately designed. The present study is valuable in the context of autism, as it could provide further support for a new disease mechanisms, and eventually indication regarding mutation screening of autistic subjects. However, unless the sample size is increased to a reasonably powered sample, the study is not conclusive and does not advance the field.

As the reviewer mentioned, the sample size was relatively small as a fellow-up study. However, we did not note in the manuscript that of 141 individuals, 88 patients were selected from 289 ASD families (72 multiplex families, 217 trios families) based on the results of X chromosome inactivation (XCI). Our previous study indicated XCI profile could be a useful criteria to prioritize families for mutation screening of X-linked candidate genes in ASD. [Gong et al. Am J
Med Genet, 2008]. RPL10, located on chromosome Xq28, is subject to X chromosome inactivation. According to this hypothesis, we selected 88 families with XCI skewing (≥70:30) for mutation screening of RPL10.

Furthermore, the CNV analysis performed after the submission of the paper was negative for the RPL10. Therefore, taken together, the absence of positive findings with mutation screening, the CNV detection and the expression study strongly suggests that RPL10 is not frequently altered in our sample.

Corrections:
1. Page 4, line 10: “Of 141 patients, 88 were selected from the families with X chromosome inactivation skewing (XCI) from 289 ASD families based on the previous study[8]. The other 53 patients were from multiplex families with random XCI.” is added.
2. Page 7, 2nd paragraph, line 4: “Our previous study indicated XCI profile could be a useful criteria to prioritize families for mutation screening of X-linked candidate genes in ASD, so we selected 88 individuals from the families with XCI skewing (≥70:30) and 53 patients from multiplex families for mutation screening of RPL10 [8]. However, we did not detect any functional mutations.” is added.
3. Page 7, 2nd paragraph, line 11: “However, we recently performed a high-throughput genotyping of 91 patients from this sample using the Illumina human1M-duo beadchip, and we also could not detect any genomic imbalance within or close to RPL10 (unpublished data).” is added.

The authors also report no significant differences in RPL10 mRNA levels, but these assays were carried out in lymphoblasts, and therefore relevance for a brain disorder is arguable.

Corrections:
1. Page 7, 2nd paragraph, line 1: “Two limitations should be considered in this study.” is changed to “Three limitations should be considered in this study.”
2. Page 7, 2nd paragraph, line 14: “Thirdly, we performed the quantification of RPL10 mRNA level in B lymphoblastoid cell lines and therefore we could have missed alterations specific to brain.” is added.

Finally, sequencing only included exons and flanking sequences, but not promoter and other regulatory regions.

Correction:
1. Page 7, 2nd paragraph, line 8: “Second, our mutation screening was restricted to exons and therefore was not appropriate to detect the presence of variants altering the expression of RPL10 in promoter regions.” is changed to “Second, our mutation screening was restricted to exons and therefore was not appropriate to detect the presence of variants altering the expression of RPL10 in promoter regions or other regulatory regions.”
2. Page 7, 2nd paragraph, line 15: “Thus, further studies on other bigger samples are warranted and the promoter regions should be investigated.” is changed to “Thus, further studies on other bigger samples are warranted and the promoter regions and other
regulatory regions should be investigated.

Reviewer 3: Dai Zhang

In the part of Methods, the authors described as “Of 141 ASD patients, 109 subjects showed mental retardation”. How did the authors identify the mental retardation? Did the authors mean that 109 patients met the diagnostic criteria of MR or these patients showed much lower IQ levels? What was the intellectual level for the 48 patients and 27 controls who received quantitative PCR examination? How could the authors exclude the mixture effect of mental retardation on the correlation between ASD and RPL10?

Autism and mental retardation can share common pathways in the pathogenesis in some degree since 75% ASD patients show low IQ less than 70. It is also reported that mutations in genes such as *FMR1* or *NLGN4* could be present in autism and/or mental retardation. Klauck et al. reported two RPL10 non-synonymous mutations, L206M and H213Q, in four boys with ASD from 2 independent families (family 42 and family 277). In family 42, both affected sons inherited the mutation L206M from the heterozygous mother. The IQ of one boy was between 20 and 34 and another was 80. In family 277, both affected sons inherited the mutation H213Q from the heterozygous mother. The IQ of one boy was between 50 and 69 and was not assessed for another boy. According to this information, the rare mutations of RPL10 seemed to be associated with autism with severe to mild mental retardation. In our screening, of 141 ASD patients, 109 subjects showed low IQ (<70).

Corrections:
1. Page 4, line 6: “Of 141 ASD patients, 109 subjects has mental retardation” is changed to “Of 141 ASD patients, 109 subjects showed low IQ (<70).”
2. Page 4, 3rd paragraph, line 1: “A sample of 48 patients (34 males and 14 females) and 27 controls (15 males and 12 females) were available for B lymphoblastoid cell lines (BLCL).” is changed to “A sample of 48 patients (34 males and 14 females, of which 35 patients IQ<70) and 27 controls (15 males and 12 females, all IQ>90) were available for B lymphoblastoid cell lines (BLCL).”

The authors sequenced all RPL10 exons and flanking junctions in 141 ASD patients, however, they did not identify any missense mutation in their patients. On the other hand, Klauck et al. had identified two non-synonymous mutations, L206M and H213Q, in the C-terminal domain of RPL10 in four boys with ASD from 345 patients. As both the two study used the European populations, what the other reasons might cause the polymorphic difference across the two samples? They authors should explore what potential factors caused this difference in the Discussion.

Correction:
Page 6, Discussion, line 8: “We performed this study in European population as well as Klauck et al [6] in order to exclude the possible sample stratification. Other factors, such as the sample size and highly genetic heterogeneity of ASD, should be considered when explaining the absence of *RPL10* mutations in our sample.” is added.
The authors mentioned that based on their sample size they had 56% of chance to detect at least one mutation in their sample. How did they get the 56%, what are the parameters for this estimation?

The possibility to find nothing is \((343/345)^{141}\).(The previous study found 2 mutations in 345 individuals). So, the possibility of find at least one mutation is \(1-(343/345)^{141}=0.56\)

What was significant level the authors set for the statistic analysis?

Correction:
Page 5, 2nd paragraph, line 2: “The significance level for all statistical tests was \(P<0.05\).” is added.

Correction:
Page 2, background, line 3: “Ribosomal protein L10 (RPL10), located on chromosome Xq28, is a key protein in assembling large ribosomal subunit and protein synthesis.” is changed to “The ribosomal protein L10 (RPL10) gene, located on chromosome Xq28, codes for a key protein in assembling large ribosomal subunit and protein synthesis.”