Author's response to reviews

Title: Evaluation of microdeletions and microduplications associated with cognitive impairment in a large cohort of subjects with autism spectrum disorders identifies duplications at 15q11-q13, 22q11 and Xp11/TM4SF2

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Author's response to reviews: see over
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To the Editors, BMC Medical Genomics:

We thank the reviewers for their constructive suggestions for our manuscript. We have revised the manuscript based on those comments. Generally, we have made a shift in the manuscript to highlight the MLPA method (plusses and minuses) and its potential use in genetics. In addition, we have examined regions in the genome that have not been a part of prior CNV analyses (particularly regions of the X chromosome), such that some of the issues of overlap are ameliorated. In two cases where we have identified a CNV that has not been extensively characterized, we now include data in controls.

A point-by-point response to the reviewer critiques is appended here.

**Reviewer 1 (Dr. James S Sutcliffe):**

1) *There is no discussion of testing or comparing control subjects for SV at the loci tested.*

In the revised manuscript, we have now surveyed controls for those variants where there was not already a strong association of a structural variants (SV) with a syndrome. The two genes/loci for which we now carry out analyses in controls are *TM4SF2* and *ASMT*. Mutations in *TM4SF2* have been implicated in cognitive impairment, but not SVs; similarly, mutations in *ASMT* have been implicated in ASD, but not SVs. The data from controls are now included, which leads to the conclusion that the *TM4SF2* duplication may be a neutral polymorphism, which is an important finding as the field was not resolved on this. In addition, the *ASMT* duplication appears associated with ASDs and merits further studies in independent samples.

2) *Have any of the samples assessed in the work been analyzed in previous studies of SV in Autism?*

Many samples tested in the current study are included in the AGRE repository although in some cases (particularly sex-chromosomes) the analyses were absent in prior reports. We now include AGRE numbers where relevant. To address the issue of overlap, we have now included additional data on the X chromosome, which has not been studied to the extent that other chromosomal regions have been. In particular, we include data on the pseudoautosomal region 1 (PAR1), which has not been systematically surveyed in ASD in any study published to date.

3) *Indicate which SV presented here has been reported elsewhere and/or is reflected in the TCAG database.*

All SV reported, with the exception of those on chromosome X and PAR1 are known genetic conditions and are part of accepted medical genetic tests. The two on chromosome X are not in the TCAG database and this has now been clarified.

4) *In this reviewer’s view, it would have been nice to see representative examples of MLPA results indicating putative SV, which was subsequently confirmed (or refuted).*
Representative MLPA results have been added as Figures 1b and 2b.

5) The rationale for the conclusion “15q11-q13 duplications, including two novel microduplications, might indicate an etiological pathway of aberrant glutamate signaling interacting with epigenetic factors in some ASD individuals” is not clear.

We have revised this text to “We did observe instances of microduplications in 15q11-q13, including two novel microduplications, and microduplications in 22q11.2, which warrant further study to discern the role of candidate genes and gene dosage alterations in those intervals.”

6) The FISH method section starts with “MLPA”.

This has been corrected.

Reviewer 2 (Dr. Zdenek Sedlacek):
1) There can be different views of the autism-MR association, but it should definitely be mentioned in the discussion.

We have now discussed the autism-MR association in the background as well as in the discussion.

2) In relation to the above, information on the prevalent MR status and MR type (syndromic, non-syndromic) of the test subjects should be added if available.

We now include data on this (in so far as it is known; only one third of the patients had a Raven IQ score available).

3) Cannot they segregate with MR - can information on the MR status of the other family members be obtained?

We agree that checking segregation with MR or with other phenotypes instead of autism would give important information. Unfortunately, these data were not available for the relevant families.

4) It might also be worth mentioning that although the study has not found CNVs in an overwhelming majority of the MR genes tested, this does not mean they are not involved - most defects previously described in these genes were point mutations not detectable using MLPA.

This has been added in the discussion.

5) The authors should soften statements like “Our findings confirm that gene dosage abnormalities involved in CI are also significant etiological factors in ASDs”.

This has been revised.

6) The authors should also comment on the diagnostic yield of their procedure - is it worth to use a similar MLPA panel in the routine examination of autistic subjects? This might be different for
established autism loci (15q, 22q) and for the other loci. Testing for known conditions is of great value in autistic patients. As very few autism-predisposing genes have been identified, only the identification of a known genetic condition allows genetic counselling and testing in the families with autism. Perhaps the focus of the paper could be shifted a bit in this direction.

Thank you for these constructive suggestions. More discussion regarding the application of MLPA method in ASDs has now been added.

7) Although individual cases with abnormal findings are discussed in detail, the manuscript lacks a discussion of the patient sample as a whole and of the method used (yield, regions detected, frequency of de novo vs. inherited CNVs etc.) from the perspective of studies of other large samples (autistic, MR, normal, ...) using different methods (subtelomere MLPA, array CGH, SNP arrays, ...). Papers like Jacquemont et al. 2006 might deserve citation. Also, any CNVs found have definitely been checked against databases of known population polymorphisms, but it is not mentioned in the manuscript.

Further discussion on all of these points is now included.

8) Minor comments:
   a) The manuscript may benefit from shortening - a modified Table 1 can be included as a regular table, and long lists of syndromes or genes/probes can be deleted from the text (p. 9, 10, 13, 14). Also the discussion of the 15q and other loci is rather long.

   We have removed the long lists of syndromes and the longer discussion points. Supplemental Table 1 is included with the syndromes.

   b) Although subjects were screened to exclude FRAXA (p. 6, presumably by FMR1 analysis (?)), the detection of FMR1 expansions using MLPA is discussed (p. 19) - are these cases not excluded from the sample?

   ASD subjects with FRAXA have been excluded as is now mentioned in the method. There are MLPA probes on FMR1 and FMR2 included in this study, but they don't allow the detection of the trinucleotide expansion as we now explain.

   c) The term "expression" should not be used in the description of genomic copy number (p. 14 line 4, p. 19 line 15).

   This has been revised.

   d) Rearranged/incomplete sentences, misprints: "...altered binding of MLPA results..." (p. 15); "... by either trinucleotide repeat expansion..." (p. 19); "D15q..." (3x, p. 8).

   We were trying to highlight that those probes are designed to detect gene dosage change but not to detect trinucleotide expansion change. We hope it is clearer in the new version of the manuscript.