Reviewer's report

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

Version: 2 Date: 31 March 2008

Reviewer: Zdenek Sedlacek

Reviewer's report:

The manuscript by Cai et al. describes the analysis of copy number variants (CNV) at multiple loci involved in mental retardation (MR) in 279 individuals with autism using MLPA. The work is a timely and valuable contribution to the mosaic of current knowledge and should definitely be published. However, the manuscript brings several questions, the addressing of which might help to improve the message of the report.

Minor essential revisions:

1) A variable degree of MR is found in a ~3/4 majority of patients with autism. The nature of the association and the functional links between these two traits remain unclear. However, these traits are separable: normal or superior intelligence is observed in a fraction of autistic individuals. As only 2/279 patients were diagnosed with Asperger syndrome, the sample examined by the authors was perhaps even more enriched for individuals with MR. It may be that the analysis of defects at known MR loci in such a sample can rather find genetic causes of MR, which are accompanied by autistic features in the particular patients. This may parallel the example of the fragile X syndrome (FRAXA), which is often associated with autism, but FMR1 does not seem to play a role in autism outside FRAXA (and FRAXA cases are usually excluded from autism patient samples). There can be different views of the autism-MR association, but it should definitely be mentioned 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.

3) The genetic defects at MR loci observed by the authors very often lack segregation with autism in the families tested. Cannot they segregate with MR - can information on the MR status of the other family members be obtained? This can be especially interesting in the case of the partial duplications of TM4SF2 (which may inactivate the allele and possibly cause MR like other mutations described in this gene), or in the case of the "silent" mutation in ARHGEF6 gene (where a variant in an individual with MR (?) not found in databases or normal controls is remarkable, especially as "silent" mutations could affect splicing, RNA stability, protein levels, conformation...).

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.

5) The authors should soften statements like "Our findings confirm that gene dosage abnormalities involved in CI are also significant etiological factors in ASDs." (Abstract, p. 4 top; also Introduction, p. 6 top; Conclusions, p. 21) - the evidence for this is not that strong.

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.

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.

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.

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?

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

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

**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.