Author’s response to reviews

Title: The clinical benefit of array-based comparative genomic hybridization for detection of copy number variants in Czech children with intellectual disability and developmental delay

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Dear Editor,

Please find attached our revised manuscript for submission to BMC Medical Genomics entitled “The clinical benefit of array-based comparative genomic hybridization for detection of copy number variants in Czech children with intellectual disability and developmental delay”.

Thank you for your valuable time and useful comments to our manuscript. We appreciate your and reviewers’ inputs which definitely help us to improve the quality of our manuscript.

Reviewer 1:

Wayhelova et al. provide interesting data on Agilent platform array results in mixed population of Czech pts with neurodevelopmental problems/dysmorphism. The choice of Agilent is reasonable, although one must bear in mind that other platforms like CytoSure from ISCA would include LP/P rearrangements of less than 200kb. It can roughly be estimated that without the 200kb threshold the additional P/LP findings could amass to about 5%. The results are not novel as they confirm the 20% additional efficiency of array after traditional cytogenetics. The added value of this work may be in already provided quite detailed phenotype-genotype analysis of cases. This could be useful for clinicians searching for clinical significance of unique rearrangements in their pts.

Our response:

We decided to unify the threshold to 200 kb for minimal size of non-polymorphic CNVs to be interpreted. Based on the novel recommendation by Silva et al. (2018) called „European guidelines for constitutional cytogenomics analysis“ diagnostic platforms should achieve a resolution 200-400 kb for postnatal referrals of developmental delay and congenital anomalies (lines 348-350, 372-373).

We provided some information regarding to OMIM „disease-causing“ genes. They are provided in attached additional files. For VOUS patients we provided some extra information concerning to patients’ phenotypes. They are provided in additional file 5 (List 1).

Major comments:

1) English could be greatly improved, e.g. '...as the most common identifiable issue...'

Our response: The improvements of English are highlighted in the revised manuscript.
2) Have there been any interesting candidate genes within unique regions that could be candidates for the observed phenotype? The content of the duplicated or deleted regions is the strongest factor for their pathogenicity. This data in the Results and Discussion could greatly improve the work.

Our response: The gene content (OMIM disease-causing genes) is mentioned in the Additional Files for every class of CNVs (the list is provided in the manuscript). For unique non-recurrent pathogenic/likely pathogenic CNVs the gene content is mentioned in the Additional File 3. As an example, we mentioned and discussed one case of patients with non-recurrent P/LP CNV in the manuscript (lines 321-330).

3) Please explain why reanalysis of random DNA samples is recommended.

Our response: The reanalysis is recommended to confirm the complementarity for different Microarray platforms and for internal quality check. More details to explaining the reanalyses are mentioned in Discussion (lines 370-375).

4) What hard data do you provide to show that CNVs less than 500kb are likely familial (not familiar) in origin? I do believe this is overestimation.

Our response: The data concerning to CNVs origin are provided in Additional Files and the outputs are commented in the manuscript (lines 343-357).

5) I would always write 'susceptibility locus' towards 15q11 and 16p13, i.e. I would never call them pathogenic by themselves as suggested in lines 275-276. Have you run WES particularly on these pts?

Our response: We accept your comment, we decided to classify them pathogenic/likely pathogenic due to their recurrence in many affected individuals, however we added “susceptibility locus” in the manuscript. We have not run WES/WGS so far, but we think about it for our future research activities. We agree that WES/WGS can be helpful approach to identify the “second hit”. The detailed explanation is provided in the revised manuscript (lines 307-313).

6) Sentence 'Most individuals...' lines 54-55 is controversial (what age?). Mild end of ID is 'school age' and this is much more common.

Our response: The corrected sentence is provided in the revised manuscript (lines 54-56).
Reviewer 2:

This is a solid piece of work based on studying the chromosome associated causes of intellectual disability in children from the Czech Republic. It contains no unexpected conclusions and agrees in almost all aspects with the results derived from similar and often much larger studies published earlier from other countries. However, the work demonstrates that care and diligence can and should be rewarded and sends important messages to other Eastern European and developing countries on what is required to establish well organized good genetic health care facilities based on molecular analysis. Another aspect that the author's might consider introducing, is information on the financial investment required, both in terms of equipment and personal.

In summary, I recommend accepting this submission for publication primarily because of the message it sends to groups in many countries that are still struggling to get themselves established in modern cytogenetic diagnosis. Some specific analytical aspects that the authors might consider further have been mentioned in the text under reviewer's comments, as well as some English suggestions.

Our response: The microarray analysis on DNA samples is a gold standard in many molecular diagnostics laboratories in Europe and the information on costs and financial investment can be found on websites of manufacturers and, on workshops and shared among laboratories. Every accredited and certified molecular laboratories providing cytogenetics and molecular cytogenetics assays should guarantee skilled and qualified laboratory staff and appropriate laboratory equipment.

We also thank for some English suggestions, we used them and highlighted in the manuscript.

Reviewer’s question on a case presented in the Figure 1: was this XY translocation identical to the rearrangement generally found in XX males and if not, in what way did it differ? Did you karyotype the mother?

Our response: The description and answer is provided in the manuscript (lines 158-167).

Reviewer’s question: Inbreeding coefficient in Czech Republic?

Our response: the answer is provided in the manuscript (line 362).

Reviewer’s question: Did you question the parents about consanguinity and is there any information in the Czech Republic about the inbreeding coefficient?
Our response: The patient’s parents are always asked concerning to their consanguinity in the course of the preliminary genetic counselling. The detailed response is provided in the manuscript (lines 358-366).

Reviewer’s comments on the use of banded karyotyping. The authors emphasis the need for continuing with a banded karyotyping procedure since array CGH cannot detect balanced chromosome rearrangements. True, but based on their own data, they detect all structural rearrangements by CGH in which a numerical imbalance occurs. This begs the question of the importance of knowing the proportion of patients in which balanced translocations or inversions occur. For the study presented here, I suspect not very much and by eliminating the banded karyotyping a lot of time and money could have been saved and diverted to carrying out greater numbers of CGH assays and their analysis.

Our response: We commented your note in the manuscript (lines 380-383).

Reviewer 3:

The manuscript entitled "The clinical benefit of array-based comparative genomic hybridization for detection of copy number variants in Czech children with intellectual disabilities and developmental delays" by M Wayhelova and co-authors describes the aCGH results of 542 patients with ID/DD, ASD and MCA. The manuscript is nicely written and results are presented in the clear way. The basic objection however, that arises after reading the manuscript is whether it really brings something new into the field. Such works have been published 10 years ago on already much bigger cohorts. However, because I think that ultimately the decision to accept this work to the Journal at the level of the impact factor above 3 belongs to the Editor of the Journal, I would like to specify some minor comments:

1) Authors used SNP array- but in the Results section the analysis is not described and the final results for LOH are not presented. Is that because the SNPs are not giving the important information? It should be discussed, because maybe the higher price for these arrays are not worth to pay if they do not bring anything into the diagnostics.

Our response: We added the results for LOH and they are provided in the manuscript (lines 215-224). The detailed description is provided in the Additional File 5, List 2) and the results are discussed in the Discussion (lines 358-366).
2) The only interested part of such publication recently can be detail description of VOUS changes. Exactly what kind of CNVs have been included in that group? Why? What kind of genes were included in these CNVs? What was the detailed clinical phenotype of these patients? These kind of information is interesting and can expand existing knowledge, can also be used for potential readers in their routine work.

Our response: The detailed information are provided in Results (lines 204-214) and their description including patients' phenotypes is provided in the Additional File 5 (List 1).

Note for Editor:

As required by Reviewers, we added some new information, which are mentioned in Additional Files (OMIM genes, phenotypes etc.). Previously submitted Additional Files were modified and new Additional Files were created to comply these requirements.

In the section “Funding” we changed the project number to MUNI/A/0958/2018, because it is a current project number at the Faculty of Science (Masaryk University, Brno).

Since all the corrections have been made, we hope the Manuscript will be accepted without any further changes. If required, we will be glad to respond any further comments and questions that you may have.

Our manuscript includes two main figures (Figure 1a, 1b and Figure 2a, 2b and six tables (provided as Additional Files 1-6).

The authors declare no potential competing interests. The authors have approved the manuscript for submission. The content of the manuscript has not been published, or submitted for publication elsewhere.

Sincerely,

Marketa Wayhelova on behalf of the authors

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