Author’s response to reviews

Title: Deletion Xq27.3q28 that includes IDS and FMR1 in female patient with global developmental delays and nonrandom X inactivation

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Author’s response to reviews: see over
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Professor Giovanni Neri
Editor, *BMC Medical Genetics*
Università Cattolica del S. Cuore

Re: Revised manuscript MS: 1711317179495498

Dear Professor Neri,

Thank you for the review of our manuscript entitled, “Deletion Xq27.3q28 that includes *IDS* and *FMR1* in female patient with global developmental delays and non-random X inactivation,” by Lauren S. Marshall, Julie Simon, Tim Wood, Mei Peng, Renius Owen, Gary S. Feldman, and Michael V. Zaragoza.

We revised the manuscript for your consideration. Please note that we changed the title to “Deletion Xq27.3q28 in female patient with global developmental delays and non-random X inactivation.” The changes made are noted by page and line numbers in the revised manuscript:

**Reviewer 1: Pietro Chiurazzi**

**Comment 1**: First of all I was surprised that the extent of the deletion (144,270,614-154,845,961) was only mentioned in the legend to Figure 3!

**Response 1**: We also agree to emphasize the extent of the deletion. We have added the deletion information to the Abstract (page 2, line 15), Background (page 3, last line), and Molecular and biochemical studies (page 7, line 5) sections.

**Comment 2**: This Figure should also show at least all those genes (of the >50 included) that are already known to cause human pathology.

**Response 2**: We appreciate your comments on this figure. In order to strike a balance between having too much and not enough information on this figure, we have revised Figure 3 to include the following genes that we focused on in the discussion (*FMR1*, *FMR2*, *IDS*, *MTM1*, *SLC6A8*, *MECP2*, *GDI1*, and *IKBKG/NEMO*). To the text, we added the list of the OMIM disease-associated genes within this region (page 7, lines 8-11).
Comment 3: The Authors emphasize the presence of both the FMR1 and IDS in the deletion interval and correctly recall that a few cases with deletions that included IDS and FMR1 have already been reported in females with developmental delay, features of Hunter syndrome, and nonrandom X inactivation of the NORMAL X chromosome. In these cases the variable phenotype is supposedly caused by the high percentage of cells lacking a functional copy of the FMR1 and IDS (and FMR2…) genes. The Authors then underline the difference of their patient who exhibits nonrandom (preferential) inactivation of the MUTANT X chromosome and (obviously) tested negative for Hunter syndrome. They also conclude that this finding “does not support a primary role of the Xq27-28 region in X inactivation”. I have an easier explanation for their observation which they unfortunately disregard throughout the paper (only generically recalling that >50 genes are included in the deletion): the deletion of their patient extends far beyond IDS including practically all Xq28 and a number of other XLMR/ID genes such as MECP2 and IKBKG/NEMO. Deletions or loss-of-function mutations of at least the latter gene are known to cause extreme X-inactivation skewage because of the negative selection of cells expressing the MUTANT X-chromosome (see some of the PubMed abstracts below for a limited example). This most likely explains the mild phenotype of the described patient and all the manuscript should be restructured to take this into account (starting from the Title that surprisingly quotes only FMR1 and IDS). Please also do not use this case to disprove the alleged (but unlikely) role of the Xq27-28 region in X inactivation.

Response 3: We kindly thank you for your review of this matter. The literature sources you provided were extremely helpful, and we appreciate you taking the time to share with us these sources that will help improve the explanation of our findings. We have reviewed the literature and added to our discussion the role that NEMO/IKBG potentially plays in preferential inactivation of the mutant X-chromosome (page 9, lines 18-24 and page 10, lines 1-4). We appreciate your feedback on this matter, and realized that we had neglected to discuss the role of NEMO on preferential X inactivation of the mutant X chromosome. In addition, we removed our previous emphasis on FMR1 and IDS by changing the title to “Deletion Xq27.3q28 in female patient with global developmental delays and non-random X inactivation.”

Reviewer 2: Maria Giuseppina Miano

Comment 1: Add a detailed map with gene annotation, by using public databases (ENSEMBLE, NCBI, UCSC, etc). Of note, within the 10,6 Mb involved in the deletion are present several disease genes that could be involved in the disease phenotype here described. Please check the appropriate references in the recent literature.

Response 1: We appreciate your feedback on this figure, and we have revised Figure 3 and added the list of OMIM disease-associated genes to the text as noted above (Reviewer 1, Comment 2).
Comment 2: They could try to identify the breakpoints and propose a genetic mechanism that may trigger the rearrangement.

Response 2: We agree that the identification of the breakpoints in the deletion region is an interesting area of future research. This may allow us to propose a specific molecular mechanism. At this time, we do not have the resources to pursue this, and we will consider it as an area of future research (page 12, lines 12-13).

Comment 3: In Figure 2, the authors show the BAC array results. But neither the FMR1 results nor the SNP array (for which gene?) results. Also the segregation in the family was missed.

Response 3: We acknowledge that the results from both studies were integral in forming our conclusion about the genetic basis of our patient’s condition. We felt that with limited space, it would be best to include this information in the text rather than create a figure with additional primary data. However, you have brought it to our attention that a table would be helpful in this situation to summary the results of these tests on both the patient and parents. The Table (page 18) demonstrates that the BAC array results were generated from the patient’s DNA and the SNP array was based on the parental DNA. Because the SNP array showed that neither parent had the deletion region we were able to conclude that the deletion was a de-novo event.

Comment 4: It is not really accurate the statement about the X inactivation and its primary role in this disease presentation. The supposed inactivation of the deleted X chromosome is not clear on which data was established. HUMARA test or FMR1 alleles? The authors must show the results obtained and to discuss them accurately, as described in several other papers describing similar genetic conditions.

Response 4: Thank you for bringing it to our attention that this was not entirely clear. We used the results of both the HUMARA test and IDS enzyme activity. We have added in more detail into the Molecular and biochemical studies section (page 6, line 18, and page 8, lines 3-4). In addition, this is shown in the new Table that summarizes the results for the patient and parents.

Comment 5: The authors must improve the discussion about the correlation genotype-phenotype. They have already considered the possibility that the deletion includes genes escaping the X inactivation but they miss to discuss in detail.
Response 5: We have added in more detail to clarify our discussion of genotype-phenotype. We have discussed MECP2 as well as other X-linked MR associated genes in our review of the deleted gene regions which includes SLC6A8, and GDI1 in addition to our discussion of FMR1, FMR2, and IDS. Based on what we have learned about genes that escape X inactivation from other published studies, it does not appear to account for our patient’s phenotype. Also, we specifically highlighted the genes implicated in our patient’s condition (IDS, MTM1, MECP2, and FMR1) to demonstrate that these are generally not expressed from the inactive X chromosome. Therefore, a deletion of these genes on the inactivated X chromosome should, in theory, not have an adverse effect (page 10, lines 20-24, and page 11, lines 10-21).

Comment 6: I'm not sure that this case represents the first described in the literature with a skewed inactivation of the mutant X chromosome. Please check accurately the literature describing both familial and sporadic rearrangement of X chromosome.

Response 6: We appreciate your feedback on this matter. We reviewed the literature [references 11-15]. Our intent was to emphasize that our case was different than previous cases of Xq27-28 deletions as our patient has inactivation of the mutant X chromosome while the other cases reported had inactivation of the normal X chromosome. We reworded the text to reflect this (Conclusions: page 2, lines 1-2, page 3, lines 1-4, and page 12, lines 9-11).

Comment 7: The authors carried out the Hunter assay. But the presenting phenotype is very different from the Hunter syndrome. I’m not sure that this approach is valid.

Response 7: Although our patient did not have the classic Hunter phenotype, we felt that based on her deletion region that Hunter syndrome may be a potential cause of her condition. This was also before we had the results of the X-inactivation studies and IDS enzyme assay. Based purely on the clinical presentation and the deletion region, we felt that Hunter syndrome was a potential cause for our patient's condition. This is because Hunter syndrome is associated with developmental delays and mental retardation, and because Hunter syndrome is usually not encountered in female patients it is likely that her physical symptoms may be very different from the classic Hunter’s phenotype seen in males. Additionally, with the potential for therapeutic management of Hunter syndrome through enzyme replacement therapy we felt that it would be most beneficial to our patient to first determine whether or not she had Hunter syndrome so that we could offer her treatment as soon as possible if it did turn out that this was the cause of her condition. We added this rationale to the text (page 8, lines 17-18).
We appreciate the comments from the reviewers. There were many excellent suggestions that we used that improved the manuscript. We hope that our revisions that include a modified Figure 3, a new Table to summarize the results, and a more detailed discussion will merit the revised manuscript for publication in BMC Medical Genetics.

Thank you for your attention to this matter.

Sincerely yours,

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