Author's response to reviews

**Title:** Deletion Xq27.3q28 in female patient with global developmental delays and skewed 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 second review of our manuscript entitled, “Deletion Xq27.3q28 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 revised the title to “Deletion Xq27.3q28 in female patient with global developmental delays and skewed X-inactivation.” The changes made are noted by page and line numbers in the revised manuscript:

Reviewer 1: Pietro Chiurazzi
Comment: I congratulate the Authors for the improvements made to their manuscript and hope that eventually a satisfactory explanation for their patient's phenotype will be identified, possibly by sequencing of the many genes included in the deletion on the normal (active) X chromosome. One minor question on Figure 3: why are some genes indicated in red and others in blue?

Response: Thank you for the positive comments, and we appreciate your question about Figure 3. The colors were used in a previous format for the UCSC Genome Browser, and now we cannot determine the significance of the colors. Therefore, we revised the figure and legend using the current format.

Reviewer 2: Maria Giuseppina Miano
Comment 1: Figure 3 can be improved adding details already available at UCSC webpage (see chrX:146,000,000-155,270,560 9,270,561 bp. at http://genome.ucsc.edu/cgi-bin/hgTracks UCSC Genome Browser on Human
Feb. 2009 (GRCh37/hg19) Assembly). The authors can easily refer to information and data annotated in this genome databank. Taken into account them they can also improve the discussion.

**Response 1**: We appreciate your feedback on this figure, and we revised Figure 3 and its legend using the current format as suggested. Using this updated information, we added the total number of RefSeq genes (113) and OMIM disease-associated genes (29) to the Results (page 6, line 7) and the Discussion (page 8, line 19). We included three additional X-linked mental retardation genes (CLIC2, HCFC1, and RAB39B) that potentially might contribute to her phenotype to Figure 3 and the discussion (page 11, line 22 and page 12, lines 5-6).

**Comment 2**: I wish underline that in literature there are several case reports describing Xq27-q28 deletion in female presenting similar or other type of diseases, such as those affecting reproductive functions, associated to incomplete penetrance due to the complexity of X-inactivation phenomena. Here few examples of crucial papers missed in the MS:


**Response 2**: We have reviewed these articles that were suggested. The Brusius-Facchin, *et al* paper describes cases of two male patients with a large deletion region including the FMR1 gene (similar to our patient) that have a more severe Hunter phenotype. This paper supports our point that the large deletion region of Xq27.3-28 and the genes surrounding the IDS locus could also be related to our patient’s developmental delays. This reference has been added to the Discussion (page 9, lines 4-6).

The Marozzi *et al*. article highlights the relation between deletions within Xq26.2-q28 region and premature ovarian failure. During our research for this paper, the association between this gene region and premature ovarian failure was noted but it was not included in our case report. In the interest of focusing our paper on the potential underlying causes of our patient’s developmental delays, we did not discuss future health complications related to this deletion region. We recognize that premature ovarian failure is a potential health problem for our patient in the future, and as such, it will be an important to keep this in mind as part of her long term care and follow-up. We have addressed this in the Discussion (page 12, lines 8-10).
Comment 3: I accept the justification of the authors about the missing data about the breakpoint identification. However, they can speculate on the origin of the rearrangement given that this region is often involved in genomic rearrangements. Several authors give an explanation for that.

Response 3: We added to the discussion (page 12, line 24; page 13, lines 1-3):
“In addition, given that this region is often involved in genomic rearrangements, we might speculate that the breakpoint region may contain highly repetitive DNA sequences that predispose to abnormal recombination (Gu et al., 2008).”

Comment 4: The authors have summarized the main results in a Table. In my opinion they are still unclear. They can improve the description adding accurate information. For the SNP ARRAY: specify the probes of the SNPs delimiting the deleted segment: NCBI name, position, etc. In the table is reported a “generic” and non specific data.

Because this in the only data demonstrating that the proband carries a terminal deletion and not an interstitial deletion, I believe that it is important to describe accurately the SNP array results and integrate them with an accurate map analysis.

Response 4: We used a BAC array to test the patient; SNP arrays were done on the parents. The parents did not have the deletion. For the proband’s BAC array results, we included the ID numbers of the specific BAC clones that encompass the deletion, CTD-3109C8 at Xq27.3 and CTD-2341N11 at Xq28 to the Results section (page 7, lines 6-7), the legend for Figure 2 (page 19), and Table (page 20).

Comment 5: For the X-Inactivation: specify the genotype of the AR polymorphic repeats and analyse the segregation along the family. Because the authors attribute to the X-inactivation a crucial role to explain the proband phenotype, I believe that it is necessary to complete this data.

Response 5: Thank you for this comment. We included in the Results (page 8, lines 5-9):
“The HUMARA X-inactivation studies showed two alleles with PCR lengths of 287 and 299 base pairs that correspond to CAG repeat lengths of 22 and 26 repeats, respectively. Methylation analysis showed that the 287 PCR fragment (22 repeats) was methylated corresponding to an X-inactivation ratio of 100:0, indicating highly skewed X-inactivation.”
We did not conduct segregation analysis; we do not have the resources to do additional family studies on a research basis. We believe that the same conclusion can be made indirectly using the results of both the X-inactivation and IDS biochemical assays (see Response 6).

**Comment 6:** On the other hand, based on their data, the authors have established that one allele of the AR locus is fully inactive and the other one is fully active (see TABLE). But they do not demonstrate if the inactive allele maps on the X chromosome carrying the deletion or maps on the health X chromosome. Because their main statement is that the deleted X chromosome is preferentially inactivated, the authors must clarify this important point improving their analysis with a segregation analysis including in the study the mother and other individuals that can help to establish the cis or trans phase of the inactive AR allele respect to the Xq27-q28 deletion.

**Response 6:** The results of the HUMARA X-inactivation study showed that one allele of the AR locus was fully active (100%) compared to the other allele. We then made the assumption that the inactive AR allele maps to the mutant X chromosome based on the results of the IDS assay. We have added into our discussion the following point that because IDS is found within the deletion region, and we assume our patient only has one copy of the gene, in order for IDS activity to be positive, it must be on the active X chromosome (see Results page 8, lines 9-13 and Discussion page 9, lines 12-17). If, however, IDS activity was negative then that would mean that the deletion region was on the active X chromosome.

**Comment 7:** Of note the authors use the expression “non-random X inactivation” and “skewed X-inactivation” to refer to the same genetic condition. The two statements have different genetic values corresponding to different range of the X-inactivation:

Given two alleles for the AR locus, A and B
- RANDOM XCI 50 (A):50 (B)
- NON-RANDOM XCI 30(A):70 (B) or 70 (A):30 (B)
- SKEWED 0 (A):100 (B) or 100 (A):0 (B)

The authors must clarify this point along the MS, also in the title.

**Response 7:** Thank you for this comment; we have made the suggested changes that included revision of the title.

**Comment 8:** In Materials and Methods is still unclear the methods applied to study XCI: by standard PCR, or by fluorescent genotyping, type of oligos, allele size, etc.

**Response 8:** We included in the Methods (page 6, lines 18-21):
“The androgen receptor locus (HUMARA) at Xq12 was used to study X-inactivation [16]. The ratio of active to inactive X chromosome was determined by PCR analysis examining DNA
methylation-sensitive restriction enzyme sites near the polymorphic CAG repeat in first exon of HUMARA [16]."

We believe that additional details are not needed since the HUMARA assay is a common X-inactivation used in many clinical laboratories.

We appreciate the comments from the reviewers. We hope that our revisions that include a revised Figure 3, additional details to the methods and results, and appropriate improvements 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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