Author's response to reviews

Title: De novo frameshift mutation in ASXL3 in a patient with global developmental delay, microcephaly, and craniofacial anomalies

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Author's response to reviews: see over
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Dear Jesus Ervin Cenzon,

Please find our updated manuscript (MS: 1799827921101669, De novo frameshift mutation in ASXL3 in a patient with global developmental delay, microcephaly, and craniofacial anomalies) and our responses to the Reviewer’s Comments (highlighted in red) below.

We thank you for your time in considering our manuscript.

Best regards,

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Reviewer #1

• Major Compulsory Revisions
1. This paper describes the identification of a novel de novo 2-bp deletion in ASXL3 in a child with HHF1, developmental delay, microcephaly, autism and dysmorphism. The work adds to the current knowledge and the spectrum of mutations for the gene in a clinical population, it is of interest and there is no major error.

• Minor Essential Revisions
2. Instead of citations 18 and 19 which were not so appropriate, it would be more useful for the authors to provide a couple of sentences about the major features of the database.
   We have added a couple of additional sentences about the database to the text.

3. Suggest revising “Conclusions” which is very lengthy. This section includes discussion on the position of the 2-bp mutation and comparison with another previously reported variant. It goes on for 2 full pages and should more appropriately be called “Discussion”.
   We have changed the heading to Discussion.

• Discretionary Revisions
4. Can the authors specify whether CPGM contain data from healthy individuals such as family members?
   Yes, the database does. We have added this to the text.
5. A figure with the gene structure showing where the deletion occurs and the hotspots, if any (according to S Table 1), would be useful.

We have added the exon number to Supplement Table 1 and added two sentences to the discussion to address this.

Reviewer #2

In the manuscript by Dinwiddle et al., authors report a new allele of ASXL3 associated with number of clinical feature in a six year old female. The manuscript is well written and data presented support the conclusions.

Minor Essential Revisions

(1) specify that this is trio analysis and not candidate gene filters.

We have clarified this in the text to state that it was a trio analysis.

(2) state that no ASXL1 mutations were detected.

We have added this statement in the text.

(3) report if any additional pathogenic do novo heterozygous mutations were detected in this six year old female sample and how they were eliminated as causative.

We have added a couple of sentences describing an additional de novo SNV discovered in VAX1 and the lack of clinical overlap of micophthalmia, syndromic type 11 which allowed it to be eliminated as causative.

(4) describe more completely whether expression pattern and function studies support ASXL3 as the causative gene.

We have added a sentence to the discussion that addresses the need for more expression and functional studies to support ASXL3 as a causative gene.

Reviewer #3

Dinwiddle et al. report a de novo frameshift mutation of ASXL3 gene in a patient with global developmental delay, microcephaly, and craniofacial anomalies by whole exome sequencing of trio. This finding is consistent with the recent other reports of mutations in ASXL3 and other ASXL family gene with similar or overlapping clinical phenotypes. Authors has done excellent work to describe the clinical features of proband in manuscript

A few specific suggestions for the revision

1) Although I agree that the case is reasonable strong to make that this is a causal mutation, it is valuable for readers if authors can describe or discuss how this conclusion is reached. In the case that the clinical phenotypes are not characteristic and there is only a single case report, this may even have more value.

We have added additional description of how the analysis was done.
2) For example, how many de novo mutations were found in the proband? Is this only one or there are additional? If there is additional de novo. Was the model of recessive inheritance analyzed?
We have added sentences describing the other de novo mutation found in VAX1 and the variants found that fit the recessive model in LYST.

3) Author mentioned ABCC8 variant, did WES also confirm this variant?
Yes WES did identify this variant and we have added a statement regarding this to the text.

4) Could authors show the Sanger sequence result for 2 bp deletion of the trio in Figure? I noted that this has been confirmed in clinical lab. Because it is heterozygous change, this will be helpful information to show reader, particular no concern for space
We have added this as supplement figure 2.

5) I understand the rationale that authors want to analyze and discuss other SNVs of ASXL3 gene. However, I am confused about the statement in conclusion, as stated “---provide evidence that rare, nonsynonymous, damaging mutations are not associated with developmental delay or microcephaly”. If this statement is valid, why should readers be convinced that ASXL3 is the cause for this patient.
We have updated the text to clarify that we believe only frameshifting and/or truncating mutations in ASXL3 are pathogenic.

Minor point:

1) Not sure that need underlies for the OMIM ID.
The OMIM ID is underlined because it is hyperlinked to the OMIM website, which may change during the editing process.

2) Are additional SNVs present in other exomes in public databases?
We show the rare SNVs in ASXL3 in Supplement Table 1 and Bainbridge et al report about SNVs in ASXL3 in other public databases as stated in the text.