Author's response to reviews

Title: Bronchial isomerism in a Kabuki syndrome patient with a novel mutation in MLL2 gene

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Author's response to reviews:

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Bronchial isomerism in a Kabuki syndrome patient with a novel mutation in MLL2 gene

Dear Editor,
We are pleased to submit a revised version of the manuscript for consideration by BMC Medical Genetics. We greatly appreciated the comments of the reviewers and revised and improved our manuscript accordingly. Below we have answered to each comment from reviewers. The modified sentences are highlighted in the text.

We hope that our manuscript is now suitable for publication.

Kind Regards.
Gerarda Cappuccio and Daniela Melis
Department of Translational Medical Science
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Via S. Pansini n. 5
Reviewer #1:

Minor essential revision

1- It would be helpful for the authors to be consistent about using certain terms, such as always using "persistent fetal fingertip pads" instead of sometimes using "persistent fetal pads"; clarifying that the eversion of the lateral third of the eyelid only applies to the lower eyelid; and ensuring that gene names are always italicized

1- We thank the reviewer for these points of clarification. We modified “persistent fetal pad” into “Persistent fetal fingertip pads” (see Abstract Section line 3, Background Section line 4, Case presentation Section line 12, Discussion Section line 15 of the revised version of the manuscript). We have clarified that the eversion of the lateral eyelid applies to lower eyelid. Genes names have been italicized.

2- In the first paragraph of the Background section, 5th line, the term "hypodonia" should be "hypotonia".

2- We thank the reviewer. We intended “Hypodontia” so missing teeth. We have corrected the spelling of the word.

3- In the clinical report, first paragraph, line 8 "trough" should be "through".

3- The word has been modified.

4- In the clinical report, second paragraph, line 5 the authors state that the physical examination revealed "dystrophia". What is this?

4- We would mean dystrophy, therefore weight-for-length below the 5th percentile. We have modified dystrophy into “His weight-for-length ratio was significantly below the 5th percentile” for clarification. (see clinical report, line 13 of the revised manuscript).

5- In the Cytogenetic analysis section, second paragraph, line 7 a period is missing at the end of the sentence "...conferred by SET domain." The authors state definitively that the mutation in their patient "...strongly affects the physiological function of MLL2...". This sentence should be qualified (unless the authors performed functional studies) by stating that the mutation "would be predicted to affect...".

5- We agree with this comment as our conclusion is not supported by experimental evidence, therefore we modified the text to better qualify this sentence as suggested by this reviewer. Now it can be read “The predicted protein lacks the most important functional domains of MLL2, particularly the plant homeodomains (PHD4-6) and the SET domains that cooperate to the
methyltransferase activity of MLL2 (Dhar S.S et al. 2013). Thus, although not experimentally investigated in this report, the detected mutation would be predicted to affect the physiological function of MLL2 altering the epigenetic and transcriptional regulatory properties of the protein”, (see Cytogenetic analysis Section lines 5-10 of the revised version of the manuscript).

7- The authors state that the MLL2 mutation cases a truncated protein to be produced. However, the other possibility is that no protein product is made due to nonsense-mediated decay. This should be acknowledged.

7- We agree that NMD might cause no protein production and this revised version acknowledges this by adding the following sentence: “However we cannot rule out the possibility that this mutation may result in the partial or total transcripts degradation due to nonsense-mediated mRNA decay (NMD) contributing to MLL2 protein haploinsufficiency.” (see Discussion section, line 5 of the revised manuscript).

8- The authors need to clarify their conclusion about when to investigate for defects of lung lateralization in individuals with Kabuki syndrome. Should it be done in all individuals with this diagnosis, even if specific symptoms are not present? Should it be done in all those with predicted loss of function mutations in MLL2? Should it be done in all affected individuals with recurrent pulmonary symptoms, regardless of whether there is evidence of immunologic dysfunction? Or should it be specifically investigated only if there are recurrent pulmonary symptoms without known immunologic dysfunction?

8- We appreciate this comment. We consider investigations for lung malformations appropriated only in symptomatic patients with normal immunological patterns, especially in patients carrying mutations associated with complete loss of function. In fact lung malformations are rare in KS patients and radiation risk associated with the investigation methods has to be considered. These considerations have been reported in the Discussion Section (lines 46-47), Conclusion Section (line 1 and lines 7-9) of the revised version of the manuscript.

9- The lip pits referenced in the legend to Figure 1 cannot be seen easily in the photographs of the patient. Could arrows be used to highlight them?

9- According to the reviewer’s suggestion the arrows have been added

Discretionary Revisions

1- It would be helpful for the authors to clarify whether the genitourinary malformations in their patient consisted solely of unilateral cryptorchidism in a newborn/one month old. Was this persistent and did it need surgical correction?

1- Cryptorchidism was monolateral and still present when he was 6-month-old. Presently he is about three years old and the cryptorchidism spontaneously resolved (see Clinical report line 21 of the revised version of the manuscript).

2- The authors discuss the hypoglycemia found after birth. How low was the blood glucose level? What interventions were needed and how quickly did it resolve?
2- We are grateful for this comment. We have added the requested information in the Clinical report section, lines 6-7 of the revised version of the manuscript.

3- The authors give no further growth parameters for their patient, aside from the growth parameters at birth. It would helpful to know the growth parameters at older ages, particularly the head circumference, as the authors continue to reference microcephaly as a finding in their patient.

3- Answer: Auxological parameters at the age of 6 months have been added in Clinical report section, line 20 of the revised manuscript.

4- The authors state that left isomerism can be associated with abdominal abnormalities, including asplenia and polysplenia. They then state that an abdominal ultrasound in their patient was normal. It would be helpful to state more specifically if the spleen appeared normal on this imaging study.

4- We have specified that neither asplenia nor polysplenia were detected (see Clinical report section, line 17 of the revised manuscript).

Reviewer #2:

Major Compulsory Revisions

1- The English used for this report is acceptable. However, in some areas of the manuscript, it could be improved.

2- We thank the reviewer. English language has been reviewed by an expert.

2- In the background section of the abstract, I suggest using "intellectual disability" instead of "mental retardation".

2- We appreciate the suggestion and modified the paper accordingly.

3- Please use "persistent fetal fingertip pads" throughout the manuscript. Be consistent with it.

3- We have modified the manuscript as requested. (see Abstract Section line 3, Background Section line 4, Case Section line 12, Discussion Section line 15 of the revised manuscript).

4- Make sure that throughout the manuscript the term "eversion of the lateral third of the eyelid" applies specifically to the lower eyelid as it is not used in a consistent way.

4- We have specified it in the revised version of the paper.

5- Please make sure that gene symbols are italicized in the manuscript.

5- We have italicized all the gene symbols.

6- Please make sure you do a thorough spell check. There are many typographical errors. For example, in the first paragraph of the clinical report, the word "trough" should be changed to "through".

6- We asked an expert to perform a spell check.

7- It would of interest to the readers to learn about the growth parameters in this male patient. We are only provided with the parameters at birth but there is no information about growth parameters when he is seen by geneticists.
7- The auxological parameters at the age of 6 months have been reported in Clinical report section line 20 of the revised manuscript.

8- It is unclear what the authors mean by dystrophy in the physical exam.

8- For dystrophy we would refer to weight-for-length below the 5th percentile. We have modified dystrophy into the statement “His weight-for-length ratio was significantly below the 5th percentile” for clarification (see clinical report, line 13 of the revised manuscript).

9- Was there any asplenia or polysplenia on abdominal ultrasound?

9- We have underlined that neither asplenia nor polysplenia were detected (see Clinical report section, line 17 of the revised manuscript).

10. The authors state that the "mutation strongly affects the physiological function of MLL2". If so, they need to provide the laboratory evidence that they have investigated the in vitro effects of this mutation.

10 We agree with this comment as our conclusion is not supported by experimental evidence, therefore we modified the text to better qualify this sentence as suggested by this reviewer. Now it can be read “The predicted protein lacks the most important functional domains of MLL2, particularly the plant homeodomains (PHD4-6) and the SET domains that cooperate to the methyltransferase activity of MLL2. Thus, although not experimentally investigated in this report, the detected mutation would be predicted to affect the physiological function of MLL2 altering the epigenetic and transcriptional regulatory properties of the protein”, see Cytogenetic analysis section, lines 5-10 of the revised manuscript.

11- The authors also state that "the mutation causes a truncated protein". Do they have any evidence for that. How about nonsense mediated decay as an alternative mechanism?

11- We agree that NMD might cause no protein production and this revised version acknowledges this by adding the following sentence: “However we cannot rule out the possibility that this mutation may results in the partial or total transcripts degradation due to nonsense-mediated mRNA decay (NMD) contributing to MLL2 protein haploinsufficiency.”. See Discussion section, line 5 of the revised manuscript.

12- The authors conclude that patients with Kabuki syndrome should be investigated for defects of lung lateralization. Should this be done in a subset of symptomatic patients?

12- We consider the evaluation for lung malformation appropriated only in a subset of symptomatic patients in case of recurrent respiratory infection with normal immunological parameters. See Discussion section, lines 46-47 and conclusions line 1 and 7-9.