Author’s response to reviews

Title: Congenital emphysematous lung disease associated with a novel Filamin A mutation. Case report and literature review

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Dear Editor,

With pleasure, we resubmit the revised version of our manuscript entitled: “Congenital emphysematous lung disease associated with a novel Filamin A mutation. Case report and literature review.” to be taken into consideration for publication in BMC Pediatrics

We thank the Editor and Reviewers for their comments. We have addressed these in a point-by-point fashion. All our corrections/modifications are in red and highlighted in yellow.
We wish to thank the reviewers for their constructive comments and we hope that the manuscript is now suitable for publication in its present form.

Thank you for your kind attention and consideration.

Yours Sincerely,

The authors

Reviewer reports:

Ralph Épaud (Reviewer 1): I read with interest the clinical case of Pelizzo et al. The case is well presented and accompanied by a complete review of the literature.

I have 3 major concern:

1) In the abstract, it is mentioned the term of interstitial fibrosis, but I could not find any data in the manuscript to ascertain this feature (should be delated I guess)
Thank you for your observation. We modified the text (page 3, line 45). Interstitial fibrosis was ascertained at the histological evaluation.

2) The genetic should be more precise. What is the type and the frequency of the mutation (nonsense, stof, frameshift, etc…) as well as the bioinformatic prediction of pathogenicity. Is it predicted to be benign probably damaging (please specify the software used such as SIFT, MutationTaster, Polyphen, etc.. Thank you for your comments, we added the information (page 3, lines 123-124, 133-139).

3) In the discussion part, I would have appreciated a little paragraph on lung emphysema clinical characteristics etiology (genetics?) and treatment (quiet surprising for your patient)
Thank you for your suggestion. We revised the discussion (pages 7-8).

Minor concern:

1) Why waiting so much for the surgery of congenital emphysema?
We now address this in the text (page 5, lines 101-102).

2) Surgical procedure is not clear to me. A left lobectomy is mentioned?? But also something ("limited resection") that appears to me as segmentectomy?
We now address this in the text (page 5, line 110).
3) It is likely that your genetic analysis was oriented by the phenotype. I don't think you should then mention CF of surfactant gene that do not correspond to the clinical or imaging presentation. Thank you for your observation. The initial and clinical data are not univocal. For this reason we analyzed several genes involved in pulmonary surfactant protein deficiency (page 6, lines 123-124; 129-132).

4) What is Schinzel-Giedon syndrome? Is there any reason to mention that here? Thank you for your observation. The initial and clinical data are not univocal. For this reason we analyzed several genes involved in pulmonary surfactant protein deficiency (page 6, lines 123-124; 129-132). We added the information on SG syndrome in the text (page 6, lines 131-132).

5) Figure 1:
B)b panel is a sagittal? Please use arrows to show the lesion (emphysema). We inserted the arrows and we inserted the projection.

C: explain the coloration and use arrows to show the different abnormalities. The type of coloration is now reported in the figure legend (Azan-Mallory, 10x, image a and Tenascin 10x, image b). We added arrows for clarity.

D) again, use arrows! We inserted arrows.

6) In the discussion, maybe you should focus
We revised the discussion (pages 7-8).

Nadia Nathan (Reviewer 2): The manuscript is a case report on FLNA mutation with lung involvement. These presentations are rare enough to be of great interest for clinicians in the field of rare lung diseases. The authors report a new mutation, and a largely documented review of the literature.

Despite needed modifications, the manuscript is very interesting and well written.

Major comments.
The case report is nicely described.

1/Some precisions would be appreciated in the case description:
- "After stabilization of the subject's respiratory condition..." how? Spontaneously? With medications? We added the information to the text (page 5, lines 100-101).

- "the patient shows general muscular hypotonia": is it really muscular hypotonia, were the muscles tested, or neurological hypotonia, or hypotonia due to respiratory failure and fatigue? This sentence is in opposition with a following sentence: "the patient was not suffering from any neurological symptoms at this stage". Please precise.
Thank you for your observation, we clarified this in the text (page 5, lines 94-95).

2/ The molecular finding needs some precisions:
- How important is the mosaic? - What are the molecular arguments for the pathogenicity of the identified variant? We added the information to the text (page 6, 133-139; page 7, 150-166).
- Did you perform a FLNA staining on the lung biopsy? We specified that the same mutation was in DNA from salivary and pulmonary mesenchymal stem cells (page 6, line 140)

3/ The illustration (Figure 1) needs to be improved:
- Add the first CT-scan so the reader could appreciate the child's evolution (panels Bc and Bd could be removed as they don't add substantial info to panels Ba and Bb). We revised figure 1 and we divided the images. Figure 2

- Panel C: it may be my PDF version's problem but the resolution is low and I can't see lung fibrosis on panel Ca. Could you improve the figure's resolution and add arrows? To me, on the proposed slide, alveolar simplification is not clearly seen neither. Panel Cb: the tenasin is a extracellular matrix staining that is unfrequently used. A control panel would be useful. Here again, the resolution is low and the histology seem very different from Panel Ca. Do the 2 panels show different parts of the lung biopsy (heterogeneity of the lesions?). It's unclear.

The original image had high resolution, the low resolution is probably a problem with the PDF version. Panels a and b were made with the same magnification (x10). In the slide, two close areola districts are shown, in order to evidence that the damage was diffuse. We added arrows and have modified the figure legend for clarity.
As reported by Estany et al 2017 BMC Pulmonary medicine, Tenascin C (TNC) is a large hexameric ECM glycoprotein that is specifically and transiently expressed upon tissue injury. It is activated after local injury and down-regulated when tissue repair or scarring is concluded. While TNC localization in the normal lung was un-detectable, a well-defined pattern of TNC expression was observed in histological samples. For this reason, a control panel would only be a negative control, without the presence of coloration (brown coloration). Figure 3

- Panel D: add arrows to point out PVNH We added the arrows. Figure 4

4/ Discussion
- Table 1 and a large part of the discussion (lines 12 to 46) are redundant. An abstract of the number of described cases, median age at onset, number of cases with PVNH, male/female severity, discussion of PDA association, etc … would be much more interesting. Thank you for your suggestions. We revised the discussion (page 7-8).

- Table 1: what was the ordering criteria of the Table? Pick-up one (date of publication, number of the amino acid involved, age at onset or whatever that could be logical for the reader). Precise when the patients are from the same family. And precise the [REF] of the listed articles. We revised the table using “date of publication” as ordering criteria. We indicated when the patients were from the same family and we added the ref. (Table 1).

- Table 1 and text: Gerard-Blanluet (not Blaunluet). As I understand, in this manuscript, the male twins present a severe disease. Could it be related to premature birth (precise term of birth) and BPD? The mother and the sisters carry the mutation (and PVNH) but have no lung disorder: there is an offset in the Table (also for follow up). Age at onset is birth? 24-26 weeks are the
term of gestation? Unclear and I have no access to this manuscript to better understand. As reported also by the authors, the extreme prematurity of the twins may have added confusing features. We specified that “age at onset” is age at onset of respiratory symptoms, in some cases at birth. We added the column “gestational age”. We also added the respiratory follow-up to the Table.

5/ Conclusion Early recognition with chest imaging … this is too much fuzzy. Please precise what the reader should look for on chest imaging that should make him think about FLNA? Same remark in the abstract.

Minor comments:
Case presentation
- remove () "on the basis of radiological findings" We removed the sentence.
- "the patient shows general muscular (and not muscolar) hypotonia" We revised the text.
- 3,140 grams or 3.140 kg We revised the text.
- surfactant (the "t" is missing) We corrected this.
- Was TBX4 mutations excluded (hips and lung alveolar simplification)? The TBX4 mutation was tested for, and it was negative.

Discussion
- line 5: multiple organs ("s") We corrected this.
- One says that male FLNA mutations are more severe than in females. This has been discussed and controverted by other reports involving females. This could be better resumed in the discussion section. We added the sentences to the discussion.
- MSC: explicit We specified this.
Other changes to the discussion were also made according to the reviewer’s comments.

Table 1 We revised the Table.
- PDA: explicit under the Table. When PDA, precise if the patients are prematurely born? For clarity, we added prematurity when the information is reported in the original text.
- p.(…) or (p….). Une the same layout We used the same layout.
- Precise the type of mutation (frameshift, nonsense, splice etc…) We indicated the type of mutation.
- 5th column: chest CT scan, and avoid ";" with nothing after We corrected this.
- Line 3: dysplasia ("a" is missing), and precise the lung status at follow-up We corrected this and added the respiratory follow-up.
- Line 8: Focal hyperinflation with minimal patchy atelectasis: move to CT findings we revises the table
- Line 9: death at what age? We added that the patient died at 15 months.
- Line 10: meconium (no "h") We corrected this.
- Line 11: hyperinflation and hyperluency: is it a mosaicism aspect? Central PA enlargement: where there clinical or echographic signs of PAH? We specified that in all patients PAH was diagnosed.
- Line 12: your case: add tracheostomy in the surgery column? We added this.
Russell J. Ferland (Reviewer 3): This is a case report that details a new pathogenic FLNA gene mosaic variant (c.7391_7403del;p.Val2464AlafsTer5) in a male who developed pulmonary emphysematous lesions and perivascular and interstitial fibrosis. The case is straightforward and I only have minor issues which would aid in assessing the manuscript.

Were there other mutations identified in other genes with the genomic sequencing and how were these excluded as not being implicated. A table of such information would be useful.

We added additional information to the text (page 6, lines 133-139) and table.

The letters in the actual figures are very hard to see and there appears to be mislabeled letters in the text (figure legends). It would also be helpful to have leaders pointing to the imaging defects in the figure. We revised the figures for clarity and we divided the images.

How was it determined that there were mosaic variants (were the parents sequenced)? We revised the text for clarity (page 6, lines 133-139).

Was the lung biopsy ever assessed for sequencing of FLNA or cDNA?
We specified that the same mutation was in DNA from salivary and pulmonary mesenchymal stem cells (page 6, line 140)

Lastly, human gene names should be capitalized and italicized, and protein names being only capitalized. We revised the text.