**Reviewer’s report**

**Title:** Identification and in silico characterization of a novel p.P208PfsX1 mutation in V-ATPase a3 subunit associated with autosomal recessive osteopetrosis in a Pakistani family

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**Reviewer:** Petra Henning

**Reviewer's report:**

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Title: Identification and in silico characterization of a novel p.P208PfsX1 mutation in V-ATPase a3 subunit associated with autosomal recessive osteopetrosis in a Pakistani family

Authors: Muhammad Ajmal, M.Phil; Asif Mir, Ph.D; Sughra Wahid, M.B.S.S; Chiea Chuen Khor, Ph.D; Jia Nee Foo, Ph.D; Saima Siddiqi, Ph.D; Mehran Kauser, Ph.D; Salman Akbar Malik, Ph.D; Muhammad Nasir, Ph.D

This manuscript describes the identification of a novel mutation in the V-ATPase a3 subunit associated with autosomal recessive osteopetrosis. The clinical features are described and a disease causing mutation is identified. The authors also perform a range of in silico analyses.

Since I am not familiar with protein modeling and in silico analysis I cannot review that part of the manuscript. That part needs to be review by a second reviewer.

**Major comments:**

The authors should if possible give a more detailed clinical description. The authors claim that there was significant variability in the severity of the clinical manifestation but it is not completely clear in what aspects. Both individuals were clinically examined during the first five months and it would be helpful to know which differences were seen then. Could the differences be explained by additional pathogenic variants?
Some kind of radiological investigation (X-rays, MRI, CT-scan) to show the osteopetrotic phenotype should be included. I assume some kind of radiological examination has been performed since individual IV:1 is claimed to have "thick, dense skull" and individual IV:3 has "thick calvarium". How was this determined?

Although it is very likely that the mutation results in defective resorptive function of osteoclast it has not been directly investigated in this study. The manuscript would be greatly improved if in vitro studies of patient's cells could be performed, but I understand this might not be possible.

Specific points:

1. The resolution of several of the figures are very low in the pdf. This has to be improved for publication.

2. Osteoclastic resorption of bone occurs in the resorptive lacunae and is not mediated by general extracellular acidification. This should be explained better.

3. The reference list includes very many reviews. It should be possible to select one or two recent reviews. Also, the authors do not refer to one of the most recent and complete reviews on osteopetrosis by Sobacchi et al in Nature Reviews Endocrinology, 2013.

4. The abbreviation ARO should be explained at first occurrence in the text.

5. Why would the Allele-specific_R primer not give a band in the patients? The primer sequence fits with the patient sequence in fig 2B.

6. Table S2 has the same text as Table S3 but there are no highlighted green primers in Table S2.

7. I would be easier if the two additional files were combined into one document.
8. Individual IV:3 is denoted as a boy in the results, but as a girl in figure legend 1. The age and cause of death should be given for individual IV:3.

9. In figure legend 1, picture A is described to show blindness. I find it hard to judge blindness from this picture.

10. The SNX10 loci should be added to the list of known osteopetrosis loci in table S5.

11. Fig 2 needs clarification. Were individuals I:1-2 and II:1-4 also tested for the mutation? What were the results in that case? Please remove them from the figure or give the results. The Sanger sequence in panel B is not easily read after the mutation in the carrier parent. Only the wt sequence is given but both alleles are seen in the graph. As comparison, a non-carrier healthy control could be given, with the deleted nucleotide marked.

12. I think fig 3 could be removed. The number of helices, strands and coils are given in the text and the figure provides no additional information or statistics (no statistics possible).

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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