Author’s response to reviews

Title: A very early diagnosis of Alström syndrome by next generation sequencing

Authors:

Leonardo Gatticchi (leonardo.gatticchi@gmail.com)
Jan Miertus (director@genius.care)
Paolo Enrico Maltese (paolo.maltese@assomagi.org)
Simone Bressan (simone.bressan@assomagi.org)
Luca De Antoni (luca.deantoni@assomagi.org)
Ludmila Podracká (podracka12@yahoo.com)
Lucia Piteková (barbora.pitekova@gmail.com)
Vanda Rísová (vanda.risova@gmail.com)
Mari Mällo (mari.mallo@asperbio.com)
Kaie Jaakson (kaie.jaakson@asperbio.com)
Kairit Joost (kairit.joost@asperbio.com)
Leonardo Colombo (leonardo.colombo.82@gmail.com)
Matteo Bertelli (matteo.bertelli@assomagi.org)

Version: 3  Date: 24 Jul 2020

Author’s response to reviews:

Dear Editor,

We thank you for considering our paper. We thank the Reviewers for their generous and useful comments. We have now edited the manuscript to address the Reviewers’ concerns and provide a point by point response. The text has also been edited again by a native English speaking translator of scientific texts.

We filled and signed the 'Change of Authorship Form' that we will send you via email.
We stated clearly the role the funder(s) had in our study in the "funding" section of the declarations.

We designated the two authors with the same initials as LP1 and LP2 as you suggested.

All references have been now correctly formatted.

We trust the Editorial Board will now find the manuscript suitable for publication in the BMC Medical Genetics journal.

On behalf of all authors,

Yours sincerely,

Paolo Enrico Maltese, PhD

Reviewer 1:

1- Add the methods and table of filtering strategy for variant detection to manuscript.

2- What software and database did you use to analyze the novelty of mutation and which showed your mutation is pathogenic? You can refer to the article "Whole exome sequencing identified two homozygous ALMS1 mutations in an Iranian family with Alström syndrome" for more information.

ANSWER: A section describing the software and databases we used to analyze the novelty and pathogenicity of the variants has been added to the paper. We read with interest the article suggested by the reviewer but do not refer to it in the present case. However, it inspired us to add a brief comparison of the WES and the gene targeted panel approach to the Discussion and Conclusions.

Reviewer 2:

The paper describes an interesting case, since through the NGS analysis the authors reached the early diagnosis of AS in a girl of only 2 years.

Nevertheless the paper cannot be accepted as it presents serious mistakes:

1) The two mutations found in the patients are not novel: the authors did not understand that they match to c.1196_1202delCACAGGA (pThr399LysfsTer11; rs761292021) and to c.11310_11313delAGAG(p.Glu3771TrpfsTer18; rs747272625).

2) Frameshift mutations cannot be defined as mutations that produce a truncated protein, in the absence of an analysis of the protein product. This type of mutation generates a premature stop codon, which very often involves the decay of the mRNA.
3) In the description of the NGS analysis, the authors indifferently use the terms Whole exome sequencing and target panel analysis, confusing the reader.

4) The authors use the concept of "normal range" referring to cholesterolemia and triglyceridemia. This concept is obsolete.

5) The link between the identification of the mutations and the review of the clinical trials is not clear. Maybe, a review of the genetic bases of the AS would have been more useful.

ANSWER:
We are grateful to the reviewer for pointing out these mistakes, especially regarding variant nomenclature.

1) After an extensive check, we confirm that:

   • the first variant is c.1196_1202delCACAGGA, p.(Thr399Lysfs*11), rs761292021 as indicated by the reviewer, AND NOT c.1194_1200del: p.(Trp399Argfs*12), as indicated in our paper. It does not seem to be a novel variant, since its pathogenicity in Alstrom Syndrome is reported in ClinVar. It was published very recently and was new at the time of writing our paper.

   • the second variant is c.11310_11313delAGAG, p.(Glu3771Trpfs*18), rs747272625, not c.11309_11312del: p.(Thr3770Ilefs*21) indicated in our paper. This variant was published with another name, i.e. c.11316_11319delAGAG, p.(Glu3773Trpfs*18), by Marshall JD et al. (2015) Hum. Mutat., volume:36, issue:7 and can also be found in other papers with different nomenclatures.

   Unfortunately, we relied on the mutalyzer/name_checker software (https://mutalyzer.nl/name-checker) to verify the variant names, but it does not seem to give correct results for the ALMS1 variants.

2) We fully agree with the reviewer and have specified that these variants that generate a premature stop codon are likely to cause mRNA decay.

3) The term Whole exome sequencing has been changed throughout the text.

4) We agree with the reviewer and have deleted reference to the normal ranges of cholesterolemia and triglyceridemia.

5) We accept the reviewer’s suggestion and have deleted the section reviewing clinical trials.

Reviewer 3:

This is a well summarised review, narrated in context of a clinical case. It identifies a novel mutation in ALMS1 gene and serves as a good review of the condition. The introduction is well written and summarizes this condition well in few words. The discussion subsequently dwells into the new variants identified and challenges in diagnosing alström syndrome.

I have some minor issue that authors may wish to consider
1) From an ethics perspective, I note that parents signed a informed consent but considering that this a rare disease and the only case in slovakia, authors need to be aware that anything written in this review can easily be traced back to the patient.
2) The lines 189 to 193, could help with rewriting to convey the message more clearly.
3) Far too much of the discussion dwells on investigational medicinal products that are still unproven in terms of clinical benefit. Further trials are underway but clearly we are still in phase II of most studies. Why not spend some time discussing life style modification's and use of standard established treatments for individual organ dysfunction eg good diabetes control, managing hypertriglyceridemia, ACEi for LV dysfunction.
4) As well outlined by the authors, eye symptoms on most occasions is what tends to be what draws attention to the diagnosis in Asltrom even in our experience. I wonder if authors would consider exploring in more details some of the diagnostic challenges related to the condition.

Answer: Thank you for your appreciation to our work.
1) What the referee writes is true, so we decided to delete the origin of the patient from the text.
2) Thank you for your suggestion, we have rewritten the paragraph to make it clearer.
3) We decided to delete the paragraph on clinical trials in the discussion and have added the information suggested by the reviewer.
4) A brief sentence on the diagnostic challenges of AS has been added as suggested by the reviewer.