Author's response to reviews

Title: Exome sequencing identifies a novel mutation in PIK3R1 as the cause of SHORT syndrome

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Author's response to reviews: see over
Dear Editor:

We are very grateful for the revision of our manuscript entitled “Exome sequencing identifies a deletion in PIK3R1, causing a severe form of SHORT syndrome” (MS ID: 1901502901112736) by Barcena et al. We also acknowledge the positive comments raised by the expert who has reviewed our paper, as well as the opportunity you have given us to revise our work in response to the criticisms of the reviewer and the editor.

We have made all the changes suggested in our manuscript, revising all the linguistic comments. Also, we have eliminated from the title as well as from the manuscript itself the references to the severe form of SHORT syndrome presented by Patient 1. We still suspect that Patient 1 has a severe form of the disease compared to most of the patients published, but we are definitely based on our proximity to him and our deep knowledge on his health status more than on tangible clinical results. That is why we agree that it is more accurate to eliminate the references to a “severe form” of the disease. Also, we have removed the reiterative references to the publication of the 3 papers of AJHG during the preparation of this manuscript, mentioning it only in the Material and Methods, as we think that it is important to justify the decision of carrying an exome sequencing analysis (something completely unnecessary if the genetic cause of SHORT syndrome had already been published). We want to emphasize that our paper was written last summer, but it underwent a series of unfortunate problems including an unexpected and extraordinarily delayed revision process. Accordingly, we agree that to date, there is no need of repeating that the AJHG papers were published during the preparation of our manuscript. Likewise, and following the reviewer’s advice, we have now included the reference to the paper by Schroeder et al., 2013.

Regarding the more specific questions raised by the editor and the reviewer, I would like to clarify that the test for Down Syndrome that we refer to when talking about
Patient 2 is the routine triple screening test, measurement of nuchal translucency and serum markers. Likewise, as the data referring to the insulin resistance of Patient 2 were inaccessible, we have decided to remove that comment from the manuscript. Finally, we can now inform the reviewer that Patient 1 is being treated with glibenclamide and sitagliptin for his diabetes.

Also, we have added the e-mail address of all authors in the title page and the appropriate statement of the ethics committee in the Material and Methods section of the revised manuscript.

To close, we would like to take this opportunity to thank again our expert reviewer and the editor for both their positive comments and insightful criticisms that have allowed us to greatly improve our manuscript.

Yours sincerely,

Professor Carlos López-Otin

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**Point by point reply to the Editor’s comments:**

1. *In the introduction, the author include, among other typical features of the syndrome, low height and ocular depression. These features are already in the definition of the syndrome and should not be repeated.*  
   **Our response:** Both features have been removed from the description of other typical features.
2. In the description of patient 1 there is mention of a "reedy" voice. I do not know what reedy means. Perhaps the authors should choose a more medical term. Likewise, myopia magna sounds odd. Severe myopia is probably better.

Our response: We meant a high pitched voice. We have now changed the term “reedy” for “high pitched”.

3. In the description of patient 2 there is mention of prenatal tests for Down syndrome. What tests are the authors referring to?

Our response: We are referring to the routine triple screening test, which retrieves a probability of malformation or chromosomal syndrome by combining the values obtained from the measurement of nuchal translucency and serum markers (alpha-fetoprotein and human chorionic gonadotropin). To prevent any misunderstanding, we have now specified in the manuscript that we refer to prenatal screening tests.

Point by point reply to the Editor’s comments:

1. The writing at time is awkward. For example, the first sentence reads, “Rare syndromes are genetic disorders that affect a reduced number of individuals in the world”. Firstly, a rare syndrome does not have to be genetic. Also by stating “rare”, the frequency, by definition, is low. If you add all the rare syndromes together, there are actually a great number of individuals worldwide affected with rare disease. I would stress a rewrite of this sentence and other sentences that are similar.

Our response: We agree that it can be a confusing sentence. It has been modified, removing the adjective “genetic” and specifying that it is individually that these syndromes are scarce.

2. I would strike “severe” from the title. The patient has SHORT and seems similar to other published cases (for example, see Chudasama et al; family 1).

Our response: When comparing the case of Patient 1 with the other patients recently reported in the literature, we really think that his phenotype is more severe. For example, in Family 1 from the mentioned Chudasama et al. paper,
one of the members is 79-year-old, the lowest BMI they present is 15.1, and all patients have normal cholesterol levels. In contrast, our Patient 1 has a BMI of 11.8 at 31-year-old, and currently exhibits a poor health situation, which may compromise his options to reach a long lifespan. He also has high cholesterol, insulin resistance and diabetes, Rieger anomaly as well as glaucoma. He has almost all the possible features of the syndrome, whereas in the other reported patients they generally present some of them. But, we agree that we are based more on our close relation to Patient 1 and our knowledge about his intimate health status than on tangible clinical data. Also, we are aware that some of the patients published are still too young to confirm that they will not present also all the features of the disease. Accordingly, and following the reviewer’s advice, we have changed the title of the manuscript and removed the references to “a severe form of SHORT syndrome”.

3- I would edit/delete the conclusion at the end of the second paragraph, ie “...we conclude that this deletion causes the development of a severe form of SHORT syndrome...”. There are other cases reported with a truncated protein and the phenotype in these cases has not been noted to be dissimilar to those cases with the recurrent missense mutation. The inference that there is a genotype:phenotype correlation is premature and would need a meta-analysis of all known cases to be performed versus the 1 case reported here. As mentioned, I am not convinced that this case is any more severe than other published cases.

Our response: As answered in the previous point, we agree that a deeper analysis would be needed to do this correlation. That conclusion has been modified.

4- The authors state several times, “in preparation of the manuscript” other studies were published. I do not think they have to repeat this as it may actually be doing the group a disservice. The 3 AJHG papers were published in July of 2013 and 8 months have now passed and another 4th paper published by Schroeder et al., 2013. If they must, I would strongly suggest that they state this 1x in the Material and Methods as it does speak to their choice of sequencing technology.

Our response: We have removed that statement, leaving it only as an explanation of the decision of performing an Exome Sequencing analysis in the Material and
Methods. This paper was prepared last summer, just by the time the 3 papers of AJHG were published. Due to an unexpected and unfair setback with the patient published by Schroeder et al. (first included in this paper) followed by an extremely long revision time, a lengthy time has passed since the manuscript was prepared. Now, we fully agree with the reviewer that 8 months later, it is definitely unnecessary to repeat that the AJHG papers were published during preparation of our manuscript.

5- The authors should cite the 4th paper by Schroeder et al., especially given the presence of the pulmonary stenosis.
Our response: We have included this reference. When this manuscript was first submitted, the paper by Schroeder et al. was still unpublished.

6- I would like to know how Patient1 is being treated for their diabetes, ie how severe is his insulin resistance.
Patient 1 is now being treated with: Daonil (Glibenclamide 5 mg), half tablet three times a day before meals, and Xelevia (Sitagliptin 100 mg): one tablet every morning. By following this regime, Patient 1 is currently controlling quite satisfactorily his diabetes.

7- How was Pt2 assessed for insulin resistance at 6 months of age, and what were the lab values (if any).
Our response: After trying to contact the doctors who diagnosed Patient 2, we have found these data unavailable. As this is an information which does not compromise the diagnosis, we have decided to remove it from the manuscript to avoid any uncomfortable confusion with his doctors.