Author’s response to reviews

Title: Whole Exome Sequencing Identified a Novel Truncation Mutation in the NHS Gene Associated with Nance-Horan Syndrome

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Dear Editor-in-Chief,

Thank you for taking the time to review our manuscript entitled “Whole Exome Sequencing Identified a Novel Truncation Mutation in the NHS Gene Associated with Nance-Horan Syndrome”. We appreciate the reviewers’ commentary and suggestions very much. Per editor’s request, the current revision has been modified to address all of the comments from reviewers, and changes are highlighted using ‘track changes’ in the manuscript. Detailed point-by-point responses are given below.

Thank you and best regards,

Xue Zhang, Ph.D.
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Editor Comments and Author Responses:

Editor Comments to the Author:

The reviewers have raised some serious issues with the manuscript in its current form. Several of these issues include:

(1) insufficient description of the clinical data, which raises the question about the severity of NHS in this family.

Response: We thank the editor’s comments. We proceeded a callback survey on the families, and additional dysmorphisms of the affected patients had been provided in the manuscript in page 5, line 1-16 and page 6, line 1-2. Both of the affected individuals present long-narrow face, blepharophimosis, prominent nose and large anteverted pinnae ear, screw-driver like incisors and diastema, mild mulberry like molars, and malocclusion, and one missing maxillary second molar in the proband. The photo evidences were provided in Figure 1 and Supplementary file 1.

(2) a less than exhaustive investigation of the possible genetic variants contributing to the observed clinical manifestations, which raises the question about the likelihood of the reported variant to be ‘the’ causal variant,

Response: We appreciate the editor’s comments. We re-analyzed the WES data and the 129 candidate variants were re-interpreted according to the ACMG guidelines (line 8-21 in page 4). And the updated strict procedures were listed in Figure 3. Five of the variants were predicted to be pathogenic, however, only NHS gene was reported to be associated with bilateral congenital cataracts and facial dysmorphism, which was included in Nance-Horan Syndrome. We provide the detailed data in Supplementary file 2. Additionally, the presence of a variant pathogenic segregating in the family verified the possibility of the disease-causing mutation.
and taken together, issues (1) and (2), lead to the genotype-phenotype correlation conclusion to be weak, at best.

Response: We thank the editor’s comments. We supplemented the photos of the molars dysmorphism of the proband and affected individual II:5. Meanwhile, we explored a more exhaustive investigation of the candidate genetic variants contributing to the observed clinical manifestations. Since the individuals were mild affected, we changed the statement to be more humility to present the genotype-phenotype correlation in the manuscript (line 15-19, page 4; line 9-15, page 9).

(4) The quality of the English is poor, which limits the readability and clarity of the manuscript; therefore, the manuscript needs to be proof read for English and grammar before re-submission. All other comments from the reviewers need to be adequately addressed before this manuscript can be considered for publication.

Response: We appreciate the editor’s comments and suggestions. In order to avoid misleading the readers, we have invited a native English speaker to proof the manuscript. And the grammatical and orthographic errors were corrected and highlighted using track changes in the manuscript. And all the comments from the reviewers had been adequately addressed as follow.

Reviewers’ Comments and Author Responses:

Reviewer reports:
Shiwani Sharma, PhD (Reviewer #1):

(1) In this manuscript the authors through whole exome sequencing (WES) report identification of a novel truncating mutation in the NHS gene in a Chinese family with Nance-Horan syndrome and attempt to draw genotype-phenotype correlations on the basis of reported pathogenic variants and chromosomal aberrations in this gene. Nance-Horan syndrome is an X-linked disease with variable clinical manifestations except bilateral congenital cataracts are present in all affected males. Thus molecular diagnosis provides confirmation of clinical diagnosis of the disorder. The novel mutation identified in the NHS gene in this study though may be the cause of the genetic disorder in the affected family the manuscript does not present sufficient clinical data, except for a description in the text, to demonstrate the presence of Nance-Horan syndrome
in the proband or his maternal uncle. The proband's teeth appear quite normal from the dental image shown in Figure 1. The possibility of presence of a pathogenic segregating mutation elsewhere in the genome has also not been excluded. Thus this reviewer has some doubt about the conclusion of this study.

Response: We thank the reviewer’s comments. In order to provide more detailed clinical information, we proceeded a callback survey on the families, and additional dysmorphism of the affected individuals had been provided in the manuscript in page 5, line 1-16 and page 6, line 1-2. Except the features of long-narrow face, blepharophimosis, prominent nose and large anteverted pinnae ear, screw-driver like incisors and diastema, the patients also had mild mulberry like molars, and malocclusion, and one missing maxillary second molar in the proband. The photo evidences were provided in Figure 1 and Supplementary file 1.

We re-analyzed the WES data and predicted mutation c.C4449G, p.Tyr1483X in gene NHS probably contributed to the observed clinical manifestations. However, this is not a whole genome sequencing data analysis, so we could not deny the possibility of presence of a pathogenic segregating mutation elsewhere in the genome-wide, that associated with the observed manifestations. So, we changed the statement to be more humility to present the genotype-phenotype correlation in the manuscript (line 15-19, page 4; line 9-15, page 9).

Specific comments:

Major –

(1) Can the authors present clinical data showing facial dysmorphism in the proband and/or his uncle?

Response: Thank you. The additional dysmorphism of the affected patients had been provided in the manuscript in page 5, line 1-16 and page 6, line 1-2. Both of the affected individuals present long-narrow face, blepharophimosis, prominent nose and large anteverted pinnae ear, screw-driver like incisors and diastema, mild mulberry like molars, and malocclusion. Meanwhile, one missing maxillary second molar was found in the proband. The photo evidences were provided in Figure 1 and Supplementary file 1.

(2) If the aim of the study was to screen known cataract causing genes for a pathogenic variant in the proband then what was the premise of initially analysing the entire WES data for variants with <0.01 MAF? Why not limit the analysis to known cataract causing genes?
Response: We thank the reviewer’s questions. In the present study, the manifestations of the proband were not very typical, and we previously could not confirm that the proband had an unknown syndrome, not the cataract only. So in order to avoid of missing the underlying disease causing gene, we performed the WES data analysis with a routine filter method of MAF <0.01. And subsequent variants interpretation and pathogenic prediction had been analyzed in procedures.

(3) As the entire WES data has been analysed, do any of the other 128 potentially pathogenic variants present in the proband segregate with the disease in the family? How confident are the authors that the identified variant in NHS is the only pathogenic variant present in this family?

Response: We thank the reviewer’s comments and questions. We think the reviewer mentioned 128 potentially pathogenic variants should be 129, which were presented in Figure 3. We listed the 129 variants in an excel file as a supplementary data for review. Among the 129 variants, 5 variants were predicted to be pathogenic based on the ACMG standards and others were benign or uncertain significance. In the five candidate genes, four genes OTOA (Deafness, autosomal recessive); IGSF3 (Lacrimal duct defect, autosomal recessive); SACS (Spastic ataxia, autosomal recessive); and NPHS1 (Nephrotic syndrome, type 1, autosomal recessive) had never been reported to be associated with cataract and facial dysmorphisms and all of them are in autosomal recessive inheritance. So, considering the proband’s phenotypes and we performed the NHS gene mutation segregation analysis in the family only. we primarily predicted that mutant NHS might contribute to the pathogenicity of the patients.

(4) According to the Cat-Map database (Shiels et al, Mol Vis 2010) more than 50 pathogenic mutations including 8 chromosomal aberrations have been reported in the NHS gene. For drawing any meaningful genotype-phenotype correlations, all the reported genetic defects in the gene should be considered. Some of the cases reported in the literature to have X-linked cataract due to mutation/aberration of NHS gene also have other features such as congenital heart defects. These features should also be considered while drawing genotype-phenotype correlations.

Response: We appreciate the reviewer’s comments and suggestions. We had combined ClinVar and Cat-Map databases, and reviewed the genotype-phenotype correlations in line 15-22, page 7 and line 1-9, page 8. And no congenital heart defects was found in the affected individuals, which was described in Table 3, page 15.
(5) The Discussion could be more comprehensive and discuss the findings of the study in the context of implication of NHS gene in the syndrome including the effect of the identified mutation on the protein and its function, correlation with the phenotype and similarity/difference from other reported mutations in the gene.

Response: We appreciate the reviewer’s suggestions. And the Discussion section has been revised more comprehensive as reviewer advised in page 6 to page 9.

(6) Please correct the language and grammar in the manuscript and avoid the use of casual expressions.

Response: We appreciate the reviewer’s suggestions. And the grammatical and orthographic errors were corrected and highlighted using track changes in a whole manuscript.

Minor -

(1) Please move clinical information of the proband and the family members, and WES results from the Methods to the Results section.

Response: We appreciate the reviewer’s suggestions. We have moved the clinical information of the proband and the family members, and WES results from the Methods to the Results section in page 5-6.

(2) Please revise Figure legends so that they provide complete and accurate details about the figures.

Response: We thank the reviewer’s suggestion. And the figure legends had been revised to provide the complete and accurate details about the figures in page 15-16.

Response: We thank the reviewer’s suggestion. This is a nonsense mutation, which resulted in a stop codon, and there was no frameshift change. We have revised the mutation to be the commonly used convention of c.C4449G, p.Tyr1483X in line 14, page 2; line 13, page 6 and line 8 page 15.

(4) Please see that formatting of references conforms to the Journal style.

Response: We appreciate the reviewer’s suggestion. And the references had been edited in the Journal style in page 12-13.

(5) In Figure 2, were individuals III:2-III:5 included in the study? Why are they numbered?

Response: We thank the reviewer’s question. Individuals III:2-III:5 were not included in this study, and we have removed the number in Figure 2.

John Rosendahl Østergaard (Reviewer #2):

The authors describe a new mutation in the NHS-gene and discuss genotype-phenotype association in Nance-Horan syndrome contra X-linked Cataract.

(1) However, I do not find the conclusion: the study provided a novel insight into the understanding of genotype-phenotype association in NHS, sufficiently proven. In many aspects, the cases may not be representative and/or are not sufficiently described. For instance, as concern the teeth, a very important marker for the disease, I miss a description of the molars in all cases.

Response: We thank the reviewer’s comments and question. A detailed description including the characters of the molars of the proband and individual II:5 had been added to the manuscript, and the teeth photos had been provided in Figure 1 and Supplementary file 1. And these clinical
data showing facial and molars dysmorphism had been added in the manuscript in page 5, line 1-16 and page 6, line 1-2.

(2) Further, the affected two patients do not show any kind of mental disabilities, they have no nystagmus and were operated at an older age.

Response: Thank you. We confirm there are no mental disabilities in the two affected individuals, and both of them had cataract operation at 15 and 21 years old respectively. However, in patient II:5, he said he was previously diagnosed as nystagmus, so we corrected the manifestations in Table 1 in page 5.

(3) In addition, the heterozygous females had no posterior suture or posterior stellate cataracts. Thus, the family history show a family that is not severely affected, as also stated by the author. Such cases should be used with more humility as done in the present paper when discussing a possible differences between the NHS disease and the X-linked cataract, especially as the dental characteristics are inadequately described.

Response: We thank the reviewer’s suggestions. In this study, the affected patients showed mild dysmorphism, and we have revised the description with more humility when discussing the differences between the NHS disease and the X-linked cataract. Revision was highlighted in line 18-19, page 6 and line 8-15, page 9.