4th of April 2013

Dear Editor:

We thank you and the reviewers for the thoughtful reviews and your decision. We trust you will be satisfied with our responses (highlighted in bold) to the comments of the reviewers. We thank you for your consideration of our manuscript for publication in the *World Journal of Otorhinolaryngology*.

Please find enclosed the edited manuscript in Word format (file name: 1586-review.doc).

**Title**: *SLC26A4* mutation testing for hearing loss associated with enlargement of the vestibular aqueduct

**Authors**: Taku Ito, Julie Muskett, Parna Chattaraj, Byung Yoon Choi, Kyu Yup Lee, Christopher K. Zalewski, Kelly A. King, Xiangming Li, Philine Wangemann, Thomas Shawker, Carmen C. Brewer, Seth L. Alper, Andrew J. Griffith

**Name of Journal:** *World Journal of Otorhinolaryngology*

**ESPS Manuscript No**: 1586

In response to the Editor:

1. Please highlight the changes made to the manuscript according to the peer-reviewers’ comments

**All the changes were highlighted in yellow color.**

2. Please revise the format of authorship like the format for review articles

**We revised the format of authorship like the format for review articles.**

3. Please describe every author’s contribution to this paper. You may refer to the format.

**We added the below sentences into the author contributions section:**

**“Ito T and Griffith AG reviewed the literature and wrote the initial draft of the manuscript. Muskett J, Chattaraj P, Choi BY, Lee KY, Zalewski C, King KA, Li X, Wangemann P, Shawker T, Brewer CC, and Alper SL critically reviewed and contributed to content and revision of the article.”**

4. Please delete the extra space.

**We deleted all extra spaces before superscript showing reference number.**

5. Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

**We added the following sentences into the Core tip section:**

**“EVA is a common inner ear anomaly. We review the correlation of phenotype with genotype of *SLC26A4*. *SLC26A4* mutations are the most prevalent known cause of hearing loss associated with EVA. The number of mutated alleles is correlated with the presence or absence of a thyroid iodination defect, thyroid gland volume, severity of hearing loss, laterality (bi- versus unilateral) of the inner ear anomaly, and probability of recurrence of EVA in a sibling. We discuss the risks and benefits of genetic testing and counseling for affected patients. These concepts may be of broad interest to otolaryngologists, audiologists and other clinicians.”**

In response to Reviewer I (No. 00503663):

1. Relationships between hearing loss and MRI findings. Cambell et al described that MRI findings of endolymphatic duct and sac were associated with the degree of hearing loss. Campbell, A. P., O. F. Adunka, et al. (2011). "Large Vestibular Aqueduct Syndrome: Anatomic and Functional Parameters." Laryngoscope 121(2): 352-357.

**Thank you for this comment. We have added the following sentence that summarizes and cites the results and conclusions of Campbell et al.:**

**“Although others have reported potential correlations of radiologic findings with hearing loss phenotypes, these conclusions were based upon univariate analyses that did not account for underlying factors and correlations such as *SLC26A4* genotype, age, or other genetic diagnoses.”**

2. Air-bone gap in large vestibular aqueduct. Air-bone gap is occasionally important to suspect large vestibular aqueduct syndrome from a pure tone audiogram. Merchant, S. N. and J. J. Rosowski (2008). "Conductive hearing loss caused by third-window lesions of the inner ear." Otology & Neurotology 29(3): 282-289.

**We agree that this is a very important suggestion so we added the following new paragraph (paragraph 1) to the PATHOGENESIS OF HEARING LOSS ASSOCIATED WITH EVA section with 6 new citations/references (59 to 64):**

**“Although hearing loss is often sensorineural, bone conduction threshold testing can reveal a mixed (conductive plus sensorineural) hearing loss at low frequencies associated with normal tympanometry and middle ear findings, and an abnormal vestibular evoked myogenic potential result. These findings are thought to be due to a “third window” effect upon sound transmission within the labyrinth.”**

3. This is a review article in FRONTIERS. Future problems or future studies should be added. For example, is a next generation sequencer useful to elucidate MO mutant EVA? If so, how ?

**Thank you for this suggestion. We have added an additional section/paragraph entitled “Future Directions” to deal with this topic. Along with this change, we deleted last sentence of second paragraph on the GENETIC TESTING FOR EVA section.**

4. I could not find a reference below. Cell Physiol Biochem. 2011;28(3):545-52. SLC26A4 genotypes and phenotypes associated with enlargement of the vestibular aqueduct. Ito T, Choi BY, King KA, Zalewski CK, Muskett J, Chattaraj P, Shawker T, Reynolds JC, Butman JA, Brewer CC, Wangemann P, Alper SL, Griffith AJ. Please add this in the Reference list and proceed so as to fit to be published in Frontiers.

**We did not cite this reference because it is a review we previously wrote. We believe a scholarly review should cite original research articles as much as possible, not other reviews, especially reviews written by the authors. Although our current figures 1 and 2 were also shown in our other review, the figures are originally derived from the NIDCD website and there is no copyright. It is proper to cite the original source (the website), which we have done.**

5. Delete figures already published or obtain permission from the publisher.

**Please see our response to comment 4 regarding figures 1 and 2. Figure 3 is derived from our published JCI paper that is cited. The publisher of JCI does not require permission to reproduce figures.**

In response to Reviewer III (No. 00503805):

ABSTRACT

1. Abstract: The introduction of the terms M2, M1 and M0 is confusing. The presence of zero mutant alleles implies all normal alleles and no mutant phenotype, yet the M0 phenotype stated is still has hearing loss and aqueduct enlargement. Are the authors saying that mutations of SLC26A4 account for some but not all of the symptoms of Pendred syndrome? And that no mutant alleles of the solute carrier gene have been found for patients in the M0 group?

**We deleted the M0, M1 and M2 notation from the abstract, and revised the abstract to clarify these points.**

2. To say something does not have an independent effect is also confusing. What is the purpose of the word “independent”? Is there an effect or not?

**An independent effect means it is not an indirect reflection or effect of another underlying correlated factor. Nevertheless we have deleted “independent” to emphasize the direct correlative relationship.**

PENDRED SYNDROME (PS) AND NONSYNDROMIC EVA (NSEVA)

3. Figure 1: The panel with normal anatomy shows a cochlea with about two and a half turns which agrees with the literature. The lower left panel showing the EVA and enlarged endolymphatic sac also depicts an enlargement of the scala media, but the number of turns changed from two and a half to one and a half.

**We appreciate the reviewer’s observation. The purpose of the panel is to illustrate the relationship of the vestibular aqueduct with the endolymphatic sac and duct. We would like to avoid distracting the reader from the primary focus of the illustration. Moreover, a reduced number of turns is not always associated with EVA, and vice versa, so we have modified the legend text to:**

**“Pathologic enlargement of the endolymphatic sac and abnormal enlargement of the vestibular aqueduct are shown below. Some ears with EVA also have a reduced number of cochlear turns. ”**

CORRELATION OF SLC26A4 GENOTYPE WITH THYROID PHENOTYPE

4. The words “or both” seems out of place in the sentence beginning “Goiter, an abnormal …”

**To our knowledge, the current wording is the grammatically proper way to list two different phrases or nouns as well as the combination of the two.**

5. In the sentence beginning “Furthermore…”, consider using the words “…early adulthood…”

**We deleted the word “young adult” since early adulthood is a relative, not quantitative, description of age. Most PS-related goiter has an onset in adolescence so we feel the revised description is most accurate.**

6. As in the abstract, the introduction of the M0 group of patients is awkward.

**This is the terminology used in the publications that we are reviewing. We cannot conceive of a better way to introduce the nomenclature of M0 to describe patients with no mutations.**

CORRELATION OF SLC26A4 GENOTYPE WITH AUDITORY PHENOTYPE

7. The sentence “This developmental arrest model was refined by Sennaroglu[39].” leaves the reader hanging. The text would be improved by telling how it was refined.

**We have deleted the sentence because the following sentence and paragraph describe how this model is not applicable to understanding hearing loss caused by EVA. Given that, and the focus of our review on genetics, a longer description would be distracting.**

8. Figure 2: It is not clear what feature of the images the arrows are pointing to.

**We replaced the arrows with larger arrows to clearly point to the EVA and enlarged endolymphatic duct and sac. This is explained in the legend.**

9. As in points 1 and 6, the correlation of unilateral EVA with zero mutations of the solute carrier gene is awkward.

**We suspect the reviewer is confused why an inner ear malformation would be associated with a normal genotype result. The genotype result is only for a single gene. It only implies that *SLC26A4* mutations are not detected and the EVA is likely caused by other factors. The term “correlation” is standard medical genetic nomenclature for the association of phenotypes with genotypes.**

PATHOGENESIS OF HEARING LOSS ASSOCIATED WITH EVA

10. The second paragraph in this section introduces the Foxi1 null mouse. It would be helpful to the reader to state here that FOXI1 is a transcription factor for SLC26A4 citing Yang et al[69].

**We added the following sentences to the PATHOGENESIS OF HEARING LOSS ASSOCIATED WITH EVA section with 4 new citations/references (72 to 74, 76)**

**“*Slc26a4* is expressed in multiple nonsensory cell populations of the cochlea, vestibular labyrinth, and endolymphatic sac and duct. The *Foxi1* gene encodes a forkhead transcription factor, which regulates transcription of *Slc26a4* in the endolymphatic sac and duct, but not in the cochlea or vestibular labyrinth.”**

Thank you again for publishing our manuscript in the *World Journal of Otorhinolaryngology.*

Sincerely,

Taku Ito, MD, PhD

Andrew J. Griffith, MD, PhD

Otolaryngology Branch, National Institute on Deafness and Other Communication Disorders, 5 Research Court, Rockville, MD 20850, United States

Tel: +1-301-402-2829

Fax: +1-301-402-7580

E-mail: griffita@nidcd.nih.gov