Author’s response to reviews

Title: Histopathological and ultrastructural analysis of vestibular endorgans obtained from patients with Meniere's disease

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Author’s response to reviews: see over
We are submitting the revised manuscript entitled: “Histopathologic and ultrastructural analysis of vestibular endorgans obtained from patients with Meniere’s disease” by Andrew A McCall, Gail Ishiyama, Ivan A Lopez, Sunita Bhuta, Steven Vetter, and Akira Ishiyama, for consideration as a Research Article to BMC ENT. We would like to thank the reviewers for their insightful and helpful recommendations for the revision of this manuscript. We submitted this manuscript to BMC Neuroscience in October 2008 and have made considerable improvements to the manuscript, aligned with all recommendations of the reviewers.

**Reviewer's report**

**Reviewer #1: Toshihisa Murofushi**

*This is a morphological study of vestibular endorgans harvested from patients with Meniere’s disease at the time of labyrinthectomy. The authors described light microscopic and electron microscopic findings in these patients. They found greater changes in the semicircular canals than the utricle. While it is a weak point that there is no control material, this study may be of value.*

We appreciate reviewer’s comments. Normal age matching controls is very difficult to obtain however others and we have reported on the morphology and morphometric on normative vestibular endorgans (Lopez et al 2005 [19], Merchant 1999 [20,25], Rauch et al 2001 [26]). Thus only indirect comparisons can be made.

**Major compulsory revisions**

1. Although the authors described that significantly greater and more significantly more frequent degeneration in the horizontal and superior canals and the saccule than the utricle, the number of materials from the saccule is much limited. Therefore, the authors should only compare between the utricle and the canals.

We appreciate this suggestion. Unfortunately for technical reasons, we successfully acquired only four saccular maculae and two posterior canal cristae. We keep the photomicrographs and description of the two posterior cristae and four saccular maculae.
However, we add that the small number of saccular and posterior crista, which were acquired, limits the data. Results section of Abstract, (line 6-7) “Although only four saccular maculae were obtained, 3 out of 4 exhibited BM (basement membrane) thickening and monolayer degeneration.”

Minor essential revisions
2. In the abstract, the authors described that “Other degenerative changes were noted among the five vestibular endorgans”, they did not present any data concerning the posterior canal crista.
We have added the available data on the posterior crista. Only two were successfully acquired. We added the results and interpretation to the Abstract (line 3), the Results section (Neuroepithelial degeneration, line 12-14) “Observations from the posterior semicircular canals from two patients showed similar degenerative changes i.e. monolayer epithelialization, stromal edema and BM thickening (Fig 1C). We also have added a photomicrograph of the posterior crista (Fig 1C).

3. P.8 L.5. Prior to the first use of “BM”, the authors should explain what BM is.
We corrected this omission. We have added a short explanation of the basement membrane under Data acquisition and analysis paragraph 2, lines 10-13. “The basement membrane (BM) is a continuous network of extracellular proteins and proteoglycans located at the epithelial and mesenchymal interface of most tissues, approximately 40-100 nm thick [21]. We have recently described the composition and immunolocalization of the human inner ear basement membranes [22].”

We have added a reference, which details renal basement membrane thickening and pathology in diabetic nephropathy.


5. Fig.1 although the authors described on Fig.1-c in figure legends, there is no photo 1-(c).
We apologize for this error. Fig. 1c in the original manuscript had been removed from the manuscript. We have corrected this in the figure legends which are now correctly referred to as Fig. 1A and B. Please note that a new figure 1C has been added which is a photomicrograph from the posterior crista ampullares.

6. Fig.5 (b) “Meniere’s utricular macula” is not appropriate expression. Please revise it.
Thank you for this correction. We have replaced the wording with, “the utricular macula from a patient with Meniere’s disease”.

Reviewer # 2: Augusto Pietro Casani
Major Compulsory Revisions

The topic of this study is interesting although not clinically relevant. I have major reservations as to the study design. There was poor correlation between the clinical data and the histopathological analysis of vestibular endorgans.

We do feel that the topic of the study has direct clinical relevance. Thank you for this suggestion. We have conducted clinicopathological correlations and the results are discussed under Clinicopathological Correlations on p. 9. “Clinicopathological Correlations. All subjects except one with monolayer formation in the horizontal cristae had a significant caloric paresis, which were significantly correlated (p<0.001). As noted prior, the presence of monolayer formation was highly significantly correlated with BM thickening (p<0.001), potentially indicative of a related pathology. However, the degree of BM thickening in the horizontal crista ampullaris was not correlated with the degree of caloric paresis and the degree of BM thickening was also not correlated with length of disease prior to surgery.”

We have also added a paragraph in the discussion on p. 10. “With regard to clinical implications, the consistent finding of monolayer degeneration of the horizontal cristae ampullares in intractable Meniere’s disease is the likely explanation for the frequent occurrence of caloric paresis. Notably, the presence of monolayer degeneration was significantly associated with the presence of caloric paresis (p <0.001), and monolayer formation was significantly associated with BM thickening (p <0.001). There was not a significant correlation between the degree of BM thickening and the degree of caloric paresis. This is likely secondary to individual differences of the effect of hair cell loss on the degree of caloric paresis, or the differential effect of BM thickening on epithelial changes.”

The results demonstrated differential degrees of neuro-epithelial degeneration, with relative sparing of the utricular maculae: the saccular maculae function was not clinically examined using Vemps?

We appreciate the comment. Because our clinic had not established vestibular evoked myogenic potentials until recently, these data were not available. We added this fact on p. 10, 3rd paragraph, last sentence, “Unfortunately, vestibular evoked myogenic potentials (VEMPs), which reflect saccular function, were not available at the time that these specimens were acquired.”

The authors should explicitly indicate a correlation between histopathological results and the clinical data from patients.

Thank you for this suggestion. Based on the data available for the specimens available in this report we have expanded the description of clinical data (Table 1). We also correlated the clinical information with morphological and quantitative results. We did not found a correlation between the degree of vestibular caloric paresis and the degree of
basement membrane thickening. However, the presence of caloric paresis was significantly correlated with the presence of monolayer degenerative changes, which is reported in the results section (p. 9) and discussed in the discussion section on clinical implications (p. 10).

Reviewer # 3: Michael Strupp
This is a very carefully performed study. It improves our knowledge on the pathology of MD.
We greatly appreciate the comments by this reviewer.

1) Introduction and Discussion are a little bit too long.
We have shortened the introduction and discussion.

2) The authors might focus on their findings and discuss them.
We have reorganized the discussion section keeping focus on the discussion of our results and the implications they may have.

Minor issues:

1) Were the authors able to differentiate the damage to type I and type II cells?

We did not found differential pathology between types I and type II hair cells; we provide a description in this respect of page 8, last five lines. We are currently estimating the numbers of type I and type II hair cells in the utricular maculae acquired from patients with Meniere’s disease. Preliminarily, there is no apparent selective loss of type I vs. type II hair cells in Meniere’s disease.

2) Did the authors find differences between the anterior and posterior semicircular canals?

As described above for Reviewer 1, we have two available posterior semicircular canals, of which we have added a Fig.1C, and added to the results and discussion section. The two posterior cristae exhibited a similar morphological deterioration as the superior and horizontal canal. This is referred to on p. 8, “The findings of horizontal, superior and posterior cristae were similar, exhibiting severe degeneration to monolayer and BM thickening.”

3) Could the authors comment on the functional consequences of their morphological findings?

We have added a section on clinicopathological correlations (p. 9 in results, and p. 10 in discussion) regarding the functional relevance of the monolayer formation on vestibular physiology in the horizontal canal cristae. All subjects except one had a caloric paresis on vestibular testing indicating horizontal canal vestibular dysfunction likely related to the sensory epithelial degeneration.
4) Could the authors correlate their findings with the results of examinations of the vestibular system in patients with MD in which caloric irrigation and vestibular evoked potentials were used?

As described for reviewer 1 and 2 and based on the data available for the specimens available in this report we have expanded the description of clinical data (Table 1) and tried to correlate this information with morphological and quantitative results. We did not found a correlation between degree of vestibular caloric paresis and degree of basement membrane thickening. However, we did find a highly significant correlation between the presence of monolayer degeneration and the presence of caloric paresis. There was also a highly significant correlation between the presence of monolayer degeneration and the presence of basement membrane thickening. These have been added to the results section under clinicopathological correlations (p.9) and on p. 10 in paragraph 3, which is copied into answer to Reviewer #2, question 1.

Please let us know if you need any further information. Thank you for your consideration

Sincerely yours,

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