

Response to the comments

Dear Dr. Ma:

As we told Dr. Jin-Lei Wang (j.l.wang@wignet.com) via email on July 14, we had sent you an incomplete revised manuscript (file name: Masuda M, WJO manuscript re-1) on July 14. It was because we found one more file that asked us to correct some minor points (They are Comment #V-1-4 as described at the last part of this response letter.) after submitting the revised manuscript (re-1).

This is the complete revised manuscript. Please find it (file name: Masuda M, WJO manuscript re-2). The edited sentences are written in red in the revised manuscript.

Title: The Cause of Idiopathic Sudden Sensorineural Hearing Loss
-The Stress Response Theory-

Author: Masatsugu Masuda, Jin Kanzaki

Name of Journal: World Journal of Otorhinolaryngology

ESPS Manuscript NO: 3304

In revising the paper we addressed the comments of the reviewers as follows:

I. In addition to the reviewers' comments, we updated Table 1. Three papers investigating potential causes of ISHL have been added in the table.

Reference 1. Cho CH, Jung BS, Jung JH, Lee JH. Expression of autoantibodies in patients with sudden sensorineural hearing loss. *Ann Otol Rhinol Laryngol* 2013; **122**(2): 131-134 [PMID: 23534128]

Reference 2. Scalia G, Palermo CI, Maiolino L, Costanzo CM, Zappal D, Grillo C, Martines AM, Cocuzza S, Russo R, Serra A. Detection of serum IgA to HSV1 and its diagnostic role in sudden hearing loss. *New Microbiol* 2013; **36**(1): 41-47 [PMID: 23435814]

Reference 3. Nishio N, Teranishi M, Uchida Y, Sugiura S, Ando F, Shimokata H, Sone M, Otake H, Kato K, Yoshida T, Tagaya M, Hibi T, Nakashima T. Polymorphisms in genes encoding aquaporins 4 and 5 and estrogen receptor alpha in patients with Meniere's disease and sudden sensorineural hearing loss. *Life Sci* 2013; **92**(10): 541-546 [PMID: 23352976 DOI: 10.1016/j.lfs.2013.01.019]

II. Reviewer #503695:

II-1. Minor language polishing is needed.

The manuscript has been professionally edited by an English language improvement services.

III. Reviewer #503898

III-1. Idiopathic sudden sensorineural hearing loss is a unilateral phenomenon according to the definition. How the stress response hypothesis theory fits the presentation of unilateral phenomenon?

I appreciate that the reviewer pointed out an important issue of the stress response theory. We had already described the problem clearly in Section IV-2 (Please read the last two paragraphs of Section IV-2) and briefly in the legend of Fig. 2 of the original manuscript. To make the point clear, we described it further in the legend.

IV. Reviewer #503663

IV-1. What is the effect on the cochlear vasculatures in the stress response theory? Is NF- κ B associated or not associated with the blood vessels permeability or inflammation?

The NF- κ B activation in the lateral wall is associated with local blood vessels permeability and inflammation. This has been added in Section IV-2 as follows.

The whole systemic stressors mentioned above converge synergistically to the NF- κ B activation in the lateral wall. The NF- κ B activation initiates inflammatory responses in the lateral wall locally. **The NF- κ B-induced**

inflammatory cytokines will affect the lateral wall cell function that maintains cochlear homeostasis (Section *II-1*). The cytokines will also exacerbate inflammatory responses of the lateral wall through enhancing vascular permeability and recruitment of leukocytes (Section *II-4*) [162, 163], because the blood supply to the lateral wall is abundant. In rabbits, for example, the lateral wall contains more than 80 % of total cochlear blood [164]. The disruption of cochlear homeostasis ultimately causes ISHL. In fact, an ISHL-affected ear has high concentration of proteins in the inner ear fluid space using fluid-attenuated inversion recovery MRI [165-167], suggesting the disruption of cochlear homeostasis.

In addition to a paper by Sugiura et al. [165], five references have been added to support this

Reference 1. Gyo K. Experimental study of transient cochlear ischemia as a cause of sudden deafness. *World Journal of Otorhinolaryngology* 2013; 3(1): 1-15 [PMID: 0000000000 DOI: 10.5319/wjo.v3.i1.1]

Reference 2. Yoshida T, Sugiura M, Naganawa S, Teranishi M, Nakata S, Nakashima T. Three-dimensional fluid-attenuated inversion recovery magnetic resonance imaging findings and prognosis in sudden sensorineural hearing loss. *Laryngoscope* 2008; 118(8): 1433-1437 [PMID: 18475208 DOI: 10.1097/MLG.0b013e318172ef85]

Reference 3. Ryu IS, Yoon TH, Ahn JH, Kang WS, Choi BS, Lee JH, Shim MJ. Three-dimensional fluid-attenuated inversion recovery magnetic

resonance imaging in sudden sensorineural hearing loss: correlations with audiologic and vestibular testing. *Otol Neurotol* 2011; **32**(8): 1205-1209

[PMID: 21921851 DOI: 10.1097/MAO.0b013e31822e969f]

Reference 4. Wang Y, Yu C, Pan Y, Li J, Zhang Y, Ye F, Yang S, Zhang H, Li X, Liang G. A novel compound C12 inhibits inflammatory cytokine production and protects from inflammatory injury in vivo. *PLoS One* 2011; **6**(9): e24377 [PMID: 21931698 PMCID: 3169595 DOI: 10.1371/journal.pone.0024377]

Reference 5. Maruo N, Morita I, Shirao M, Murota S. IL-6 increases endothelial permeability in vitro. *Endocrinology* 1992; **131**(2): 710-714 [PMID: 1639018]

IV-2. The reviewer requires more detailed explanation about the organ of Corti in the stress response theory.

The following points are now described in Section IV-1.

In their paper published in 2005, they speculated that osmotic stress-induced NF- κ B activation within the supporting cells may be an important mechanism causing ISHL in addition to the activation in the lateral wall [52]. However, using a sophisticated animal model in 2009 [138], they demonstrated that cells of the organ of Corti and spiral ganglion were remarkable for the lack of NF- κ B activation by systemic inflammatory stress. On the other hand, type II fibrocytes in the lateral wall predominantly

showed the activation. The lateral wall plays an essential role in maintaining the cochlear homeostasis. In addition, NF- κ B is a well-known transcription factor that directly leads to inflammatory cytokine production, and it was observed in animal and human lateral walls, but not in the organ of Corti. Conclusively, the original hypothesis by Adams et al. is that ISHL is the result of the stress response of the cochlear lateral wall through NF- κ B activation responding to the systemic stress and dysfunction of the lateral wall, and the changes of the organ of Corti cells are the secondary phenomenon to the lateral wall dysfunction.

IV-3. About Table 2. Are adrenergic and cholinergic receptors at the blood vessels or at tissues apart from the blood vessels?

The receptors were detected at tissues other than blood vessels. This fact has been added in the legend.

IV-4. Change “I review” to “We review”.

This change has been made throughout the manuscript.

IV-5. About the Abstract. Add one or two more sentences instead of the words, “We review”.

The abstract has been reorganized.

Reviewer # not provided

V-1. The authors' affiliations should be rearranged.

They have been rearranged as your instruction.

V-2. The professional title of the corresponding author should be provided.

It has been provided.

V-3. The heading of Instruction should be revised.

It has been revised as your instruction.

V-4. Numberings of headings in the manuscript should be removed.

I would be most grateful if you could give me a permission to leave the numberings, because they have an important role in the manuscript. It will

be great help for readers to understand the present review. Could you please read the following sentences for the example.

The NF- κ B-induced inflammatory cytokines will affect the lateral wall cell function that maintains cochlear homeostasis (Section II-1). The cytokines will also exacerbate inflammatory responses of the lateral wall through enhancing vascular permeability and recruitment of leukocytes (Section II-4) [162, 163], because the blood supply to the lateral wall is abundant.

V-5. The decomposable figure should be provided.

We have revised the format of the figures as the examples that you provided us. However, we can also provide Illustrator files of the figures. The instruction for authors of your journal instructs us to submit figures in Photoshop or Illustrator files in tiff, eps, or jpeg format.

We appreciate the reviewers' constructive suggestions, and hope that our revised paper is now acceptable for publication in World Journal of Otorhinolaryngology.

Sincerely,

Masatsugu Masuda, M.D., Ph.D.

A handwritten signature in black ink, appearing to read 'Masatsugu Masuda', written in a cursive style.

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