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Linking LIMK1 deficiency to hyperacusis and progressive hearing loss in individuals with Williams syndrome

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Williams syndrome (a.k.a. Williams-Beuren Syndrome) is a multisystem disorder caused by the hemizygous deletion of a 1.6 Mb region at 7q11.23 encompassing about 26 genes, including that encoding LIM kinase 1 (LIMK1). Individuals with Williams Syndrome manifest hyperacusis and progressive hearing loss, and hyperacusis early onset suggests that it could be associated with one of the deleted genes. Based on our results about the critical role of LIM kinases in the regulation of the motile responses of cochlear outer hair cells (OHC) and cochlear amplification, we propose here that a reduced expression of LIMK1 in OHC would be the major underlying cause of the hyperacusis and progressive hearing loss observed in patients with Williams Syndrome. Moreover, we propose a novel model of gain-control for cochlear amplification based on LIMK-mediated regulation of OHC's slow motility.

Key words: Williams syndrome, LIMK, hyperacusis, outer hair cell, cochlear amplification, hearing loss

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Williams syndrome (WS) is a neurodevelopmental disorder caused by a hemizygous microdeletion of approximately 1.6 Mb on the long arm of chromosome 7 (7q11.23) containing about 26 genes, including LIMK1 and elastin.[1-5] In addition to cardiovascular anomalies, moderate mental retardation and severe impairments in visuospatial processing, WS patients show oversensitivity to sounds, including hyperacusis, phonophobia and auditory fascination.[6] The early onset and pervasiveness of hyperacusis in individuals with WS suggests that it could be associated with one of the deleted genes.[7]

Hyperacusis is defined as an oversensitivity to sound associated with an excessive auditory gain because of a dysfunction in the mechanism of cochlear amplification.[8,9] Excessive auditory gain naturally implies an intact cochlear amplifier. This points at a failure in controlling the gain of the amplification mechanism, rather than a failure in the mechanism itself, as the culprit for hyperacusis in WS subjects. Audiometric studies, DPOAE and TEOAE recordings strongly suggest that the WS subjects have OHC dysfunction.[5,10-12] In addition, studies involving OHC modulation by the ipsilateral medial olivocochlear (MOC) system showed that the mechanism affected in WS subjects is a target of acetylcholine (ACh),[8] the major neurotransmitter released by efferent terminals at the base of OHCs.[13] In normal individuals, electrical stimulation of MOC fibers reduces cochlear amplification by some 20 dB, a central result to the widely accepted hypothesis of efferent-mediated protection of auditory function from noise trauma.[13,14]

The possible origin of the dysfunction in cochlear amplification causing hyperacusis in WS subjects is still a matter of debate.[5,6,10,15] Elastin insufficiency was considered a possible cause based on early reports describing the destruction of hair bundle tip-links with the enzyme elastase.[16-18] It was hypothesized that tip-links could be composed by elastin, and elastin deficiency could lead to some kind of desynchronized movement of the stereocilia and a combination of hearing loss and acoustic nerve dysfunction responsible for an altered perception of loudness in

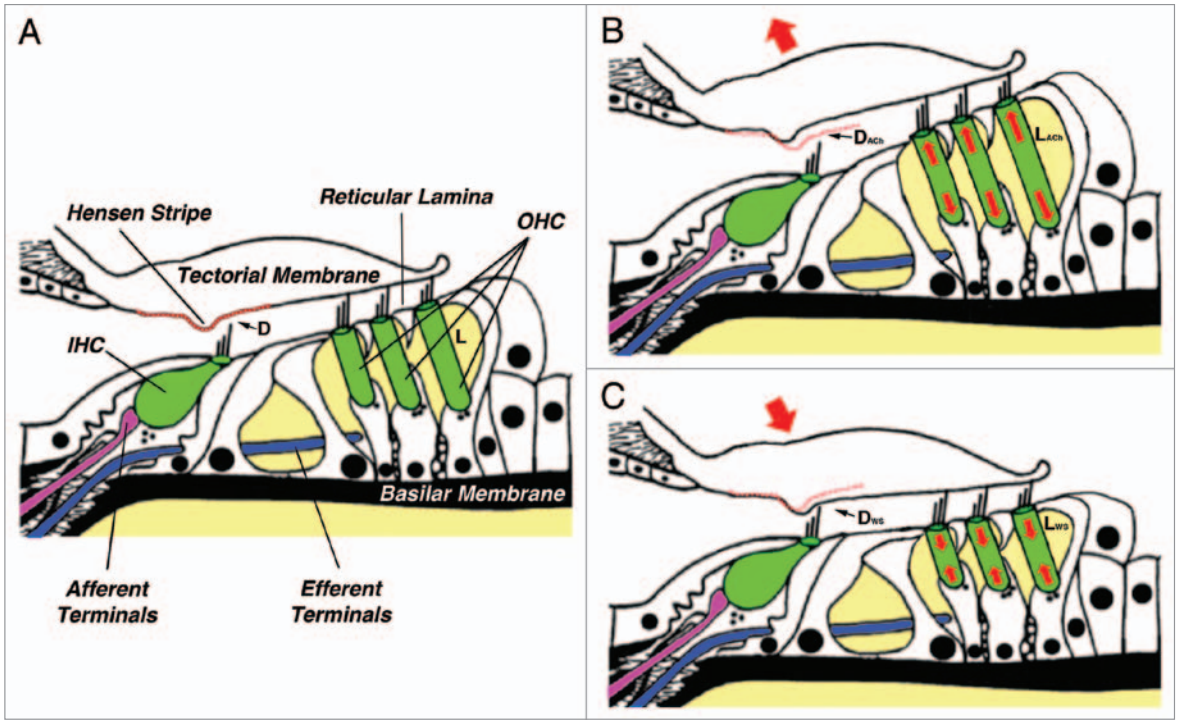

Figure 1. A novel model of gain-control for cochlear amplification based on the regulation of slow motility of cochlear outer hair cells. (A) The organ of Corti and related structures at a given steady-state condition. D, distance between the lower surface of the tectorial membrane and the tip of IHCs' stereocilia; L, total OHC length. (B) Proposed effect of ACh. OHCs would elongate (L_{ACh}>L) and D would increase (D_{ACh}>D). Auditory gain would decrease. (C) Proposed effect of LIMK1 deficiency in WS. OHCs would shorten (L_{WS}<L) and D would decrease (D_{WS}<D). Auditory gain would increase (hyperacusis). Changes in OHC length are exaggerated for clarity. See text for details.

the afferent auditory system.[7] More recent studies indicating that tip-links might actually be composed by cadherin-23 and proto-cadherin 15 make this hypothesis unlikely.[19-22]

An alternative gene that might be responsible for the auditory phenotype in WS is LIMK1, which encodes for a serine/threonine kinase that regulates actin reorganization.[23] LIMK1 gene localizes in the middle of the region deleted in individuals with WS, and LIMK1 knockout mice subjected to a fear-conditioning test showed significantly longer and more constant freezing than wild-type mice when exposed to certain sounds.[24] However, it remains unclear if that aggravated responses in KO mice were specific to auditory stimuli.[6] The gene encoding the only other known member of the LIMK family, LIMK2, localizes in human chromosome 22 (22q12) and it has not been associated with WS. To our knowledge, our recent publication is the first report linking LIMK to OHC motility and cochlear amplification, and the first suggesting that "any disruption in the signaling pathways involving these molecules could result in extreme physiological responses such as hyperacusis or deafness."[25] Thus, based on our original results, we are proposing here a deficiency in LIMK1 as the potential cause of WS-associated hyperacusis.

We found that activation or inhibition of LIMK-mediated pathways regulate cochlear amplification by increasing or decreasing, respectively, both electromotile amplitude and total length of cochlear OHCs without any effect on the performance of the plasma membrane-embedded motor (prestin) proteins.[25] Since LIMK1 is absent in OHC's stereocilia, we consider unlikely any significant effect on any active process in the stereocilia bundle putatively associated with cochlear amplification. Thus, we speculate that a deficient expression of LIMK1 would result in a simultaneous decrease of both electromotile amplitude and OHC total length. This is a very important point that reveals the fine-tuning mechanism underlying homeostatic control of cochlear amplification. Whereas OHC shortening would be associated with an increase in the gain of the cochlear amplifier (see below and **Fig. 1C**), a decrease in OHC electromotility is generally associated with gain decrease. Thus, the two OHC motile responses would be working in a dynamic equilibrium, with any change in OHC length moving the gain of the cochlear amplifier either up or down being simultaneously counteracted by a change in electromotile amplitude pushing the gain in the opposite direction.

How does LIMK1 deficiency could translate into hyperacusis? We propose the following mechanism (**Fig. 1**):

In a given steady-state condition (**Fig. 1A**), OHCs would have a particular length L and the distance between the lower surface of the tectorial membrane (TM) and the tip of the IHCs' hair bundle would have a particular value D. Sound stimulation would induce vibration of the basilar membrane (BM), generating a shearing movement between the TM and the reticular lamina that deflects OHCs' hair bundles.[26] Hair bundle's deflection would open ionic channels at the tip of the stereocilia, changing the electrical

potential inside the OHCs and triggering electromotile responses that amplify 1,000-fold and more the original amplitude of BM vibration. Since the tip of OHC's tallest stereocilia are embedded in the TM, electromotility would move up and down this structure creating a flow of endolymphatic fluid in the subtectorial space sufficient to deflect IHCs' hair bundles (which are not connected to the tectorial membrane), generating electrochemical signals that would be transmitted to the brain via the afferent terminals innervating the IHCs.[27,28]

-In the event of high intensity sound stimulation the brain would protect the IHCs by activating a negative feedback mechanism, based on the release of ACh by the MOC efferent terminals innervating the OHCs, aimed at decreasing the gain of the cochlear amplifier (**Fig. 1B**). ACh would increase the concentration of intracellular Ca^{2+}, activating Ca^{2+}-dependent, LIMK-mediated pathways targeting the OHC cytoskeleton and triggering slow motile responses leading to a significant increase in both total OHC length (L_{ACh}>L) and electromotile amplitude. The increase in total OHC length would increase the distance between the TM and the tip of IHCs' stereocilia (D_{ACh} >D), diminishing the speed of the flow of endolymph and, consequently, the deflection of IHCs' stereocilia and the gain of the cochlear amplifier.

-Genetic or physiological problems leading to total disruption or malfunctioning of the mechanism/s regulating OHC motility, such as LIMK1 deficiency in WS subjects, could be the underlying cause of hyperacusis (**Fig. 1C**). Abnormal signaling could result in excessive and/or uncontrolled shortening of OHCs (L_{WS} <L) leading to a decrease in the distance between the TM and the tip of IHCs' stereocilia (D_{WS}<D). This change in the geometry of the subtectorial space would increase the speed of the flow of endolymph and consequently, the deflection of IHCs' stereocilia and the gain of the cochlear amplifier. In addition, the displacement of the TM would close the gap between the Hensen stripe and the hair bundle of the IHCs, increasing the risk of mechanical damage of the IHC's hair bundle. This damage could be responsible for the progressive hearing loss observed in subjects with WS. Electromotile amplitude could be not affected, reduced (as in the case of LIMK inhibition or deficiency), or even increased, contributing to exacerbate hyperacusis.

Thus, the reported role of LIMK in the regulation of OHC motile responses provides a strong support to the hypothesis that hyperacusis in subjects with WS would be linked to deficiency in LIMK1. In addition, the potential involvement of LIMK1 and OHC motility in the phenomenon of hyperacusis provides a consistent and strong frame of reference for understanding the cellular and molecular mechanisms involved in the regulation of the cochlear amplifier.

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