Role of Connexin 32 (Cx32) and Hearing Loss In Charcot-Marie-Tooth Syndrome (CMTS)

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ABSTRACT
Objective: Evaluate the role of Cx32 and hearing loss associated with Charcot-Marie-Tooth syndrome using a Cx32 knockout (KO) mice model.

Study Design: Scientific Research.
Methods: Cx32 knockout mice (KO) were compared to CBA normal hearing controls and Cx32 wild type (WT) mice to evaluate inner ear development using epifluorescent microscopy and auditory performance using auditory brainstem response (ABR) testing at approximately 3, 6, and 12 months of age. Distribution of Cx32 was compared relative to Cx26 and Cx30 using immunostaining and epifluorescent microscopy.

Results: Cx32KO mice showed greater hearing loss compared to CBA controls by 4.5-6 months of age (thresholds: 51.75 +/- 10.3 dB vs 40 +/- 5 dB, respectively, p = 0.0001). The difference became more pronounced with aging (13 month CBA control threshold: 41.7 +/- 2.9 dB vs 1011 month Cx32KO threshold: 63.6 +/- 14.2 dB, p = 0.0011). Distribution of Cx32 differed from Cx26 and Cx30. Cx26 and Cx30 were prominent in the spiral lima, supporter cell region, and lateral connective tissue in the CBA, Cx32WT, and Cx32KO mice. In contrast, Cx32 showed minimal presence and solely in the supporter cell region in both the CBA and Cx32WT mice while absent in the Cx32KO mice.

Conclusion: While Cx32KO mice showed normal cochlear architectural morphology, Cx32 deletion results in earlier hearing loss. This may suggest a cochlear endolymphatic dysregulation due to its location in the supporter cell region as the cause of hearing loss in CMTS, not auditory neuropathy, as Cx32 was not present in the spiral ganglion. Cx32KO mice may serve to study hearing loss in CMTS.

INTRODUCTION

• CMTS: Multi-organ peripheral neuropathy leading to muscle atrophy/weakness, areflexia/hyopactive reflexes, and may have other associated CNS manifestations or hearing loss.1-3
• Genetics: 1 in 2,500 people have a form of the disease, X-linked form associated with over 269 mutations of Cx32 gene.1,3
• Purpose: Exact etiology of hearing loss not known, thought prior to be auditory neuropathy. Absence of Cx32 has not been studied in auditory development or auditory function.
• Cx26 and Cx30 have been well documented to be coexpressed in the spiral ganglia, and spiral ganglion. Cx26KO mice may serve to study hearing loss in CMTS.

OBJECTIVES

• Evaluate the role of Cx32 in inner ear development and function using a Cx32 knockout (Cx32KO) mice model.
• Auditory brainstem responses (ABR) were used to determine hearing thresholds.
• Immunostaining and epifluorescent microscopy were used to evaluate inner ear development and architecture, as well as its distribution in the cochlea compared to Cx26 and Cx30.

MATERIALS AND METHODS

• Mice: Cx32KO mice (n = 20) genotype verified by PCR of DNA from tail snips served as test subjects against Cx32WT controls (n = 3) and CBA normal hearing controls (n = 3). Cx32KO mice were tested between 4.5-6 months of age. Cx32WT mice were tested at 6, 8, and 12 months of age. CBA mice were tested at 3, 7, and 13 months of age.
• Cochlear & Vestibular Organ Isolation: Mice from each time period sacrificed with subsequent temporal bone harvesting and processing.
• Immunofluorescence and Epifluorescent Microscopy: Cx26, Cx30, Cx32, and DAPI staining of serial sections of cochlear and vestibular organs from the isolation process of each sacrificed mouse with subsequent epifluorescent microscopy.
• Western blot: Analysis of Cx26, Cx30, and Cx32 in the cochlea and vestibule of each mouse strain.
• Statistical Analysis: Unpaired t test with Welch’s correction.

RESULTS

Auditory Brainstem Responses:

• Cx32KO (n=20) threshold at 6.5-7.5 months (58.158 +/- 14.356 dB) was significantly higher than the Cx32WT (n=3) hearing control threshold at 8 months (45 +/- 0 dB) (t = 3.607, df = 17, p = 0.0022).
• Cx32KO (n=20) threshold at 6.5-7.5 months was significantly higher than the CBA (n=3) normal hearing control control threshold at 7 months (40 +/- 0 dB) (t = 4.976, df = 17, p = 0.0001).
• Cx26WT and CBA controls at these intervals showed no significant difference in hearing threshold (t = 2.041, df = 4, p = 0.1104).
• Cx32KO (n=20) threshold at 10-11 months (63.611 +/- 14.226 dB) was significantly higher than the Cx32WT (n=3) hearing control threshold at 12 months (45 +/- 0 dB) (t = 5.138, df = 17, p < 0.0001).
• Cx32KO (n=20) threshold at 10-11 months was significantly higher than the CBA (n=3) normal hearing control control threshold at 13 months (41.667 +/- 2.887 dB) (t = 6.110, df = 17, p < 0.0001).
• Cx32WT and CBA controls at these intervals showed no significant difference in hearing threshold (t = 1.387, df = 3, p = 0.2597).

DISCUSSION

Cx32 is not as robustly present in the Organ of Corti when compared to Cx26 and Cx30 but with more specific locality as described above. In addition, Cx32 was not detected by immunocytochemistry in the vestibular tissue although found in the cochlea supporting cell region of the basement membrane, and spiral lamina in CBA, Cx32WT, and Cx32KO mice but lesser intensity than Cx30. Green: Myosin (Hair Cells). Blue: DAPI (nuclei). Power: x10.

Immunofluorescence & Epifluorescent Microscopy:

• Red: Cx26 (Present in the lateral connective tissue, supporter cell region of the basement membrane, and spiral lamina in CBA, Cx32WT, and Cx32KO mice but lesser intensity than Cx30). Green: Myosin (Hair Cells). Blue: DAPI (nuclei). Power: x10.

• Red: Cx30 (Present in the lateral connective tissue, supporter cell region of the basement membrane, and spiral lamina in CBA, Cx32WT, and Cx32KO mice but greater intensity than Cx26). Green: Myosin (Hair Cells). Blue: DAPI (nuclei). Power: x10.

• Red: Cx32 (Present ONLY in the supporter cell region of the basement membrane of CBA and Cx32WT mice but NOT Cx32KO mice). Green: Myosin (Hair Cells). Blue: DAPI (nuclei). Power: x40.

• Cx32KO Mice: Red: Cx32 (Not present in the Cx32KO strain but present in Cx32WT and CBA mice). Green: Myosin (Hair Cells). Blue: DAPI (nuclei). Power: x10.

REFERENCES