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BASIC AND TRANSLATIONAL RESEARCH

Cochlea Transcriptome Analysis of the GJB2 gene p.V37I Mutant Aging Mice

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Abstract

Age-related hearing loss (ARHL) has been closely linked to genetic factors, with studies identifying the p.V37I mutation in the GJB2 gene as a potential contributor to ARHL. To investigate this, we generated a p.V37I mutant mouse model and performed auditory brainstem response (ABR) testing, cochlear morphology assessments, and transcriptional sequence of mutant and wild-type (WT) mice at different ages. Our results indicated that this kind of GJB2 mutation does not lead to cochlear developmental abnormalities, and aging mutant mice exhibit only mild hearing loss compared to WT mice, without significant cochlear morphological differences. However, transcriptional analyses revealed substantial differences between mutant and WT mice. GO enrichment analysis of the DEGs between aging mutant and WT mice highlights significant enrichment in biological processes related to neural and sensory functions. Notably enriched terms include "neuron-to-neuron synapse," "immune response-activating signaling pathway," "regulation of synapse structure or activity," and "sensory perception of sound." These findings suggest that the p.V37I mutation in aging mice affects synaptic and calcium signaling pathways, as well as sensory system development. Despite these molecular changes, cochlear function remains normal in early life; however, as the mice age, hearing loss accelerates, likely due to a diminished capacity for gene-mediated protection against external stimuli.

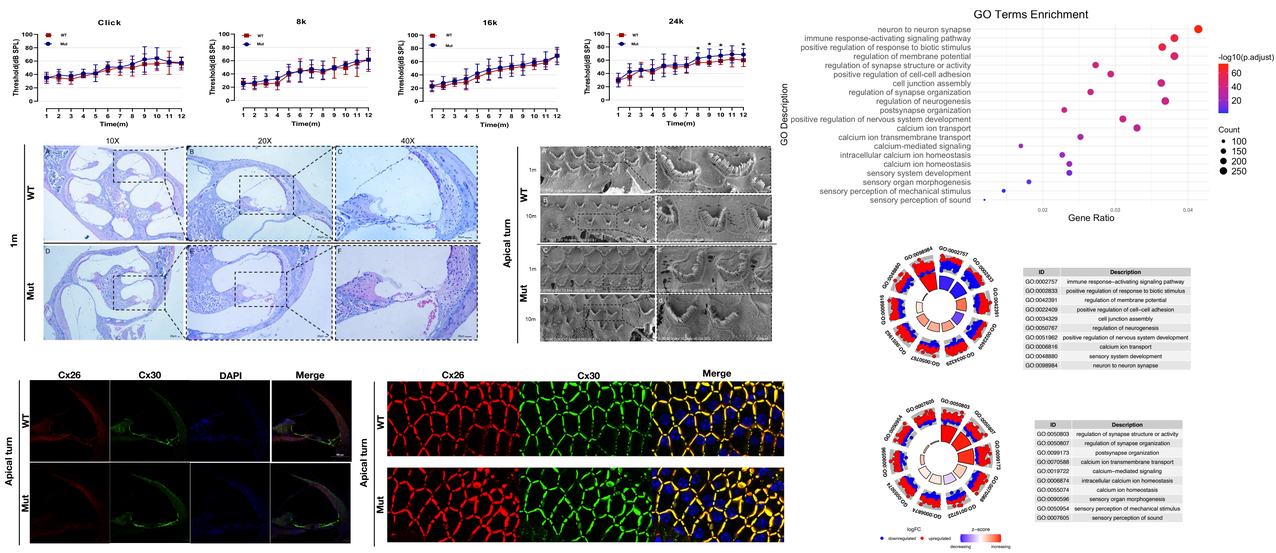
Objective

Given the substantial impact of the GJB2 p.V37I mutation on auditory pathophysiology，it is essential to develop an appropriate animal model to investigate its underlying mechanisms. We engineered a multi-base edit in mice‘s p.V37 codon of the GJB2 gene. This modification successfully replicated the human p.V37I amino acid sequence, thereby enhancing the model’s relevance for studying the mutation‘s effects, thereby potentially informing future treatment and prevention strategies.

Method

- CRIPSR/Cas9 to engineer the p.V37I mutant mouse
- H&E staining to observe the cochlear morphological changes
- SEM to observe the cochlear micromorphological changes
- IF to observe the expression of connexin 26 and connexin 30
- RNA-seq to compare the transcriptional differences between WT and mutant mouse

Result



ID	Description
GO:0002757	immune response-activating signaling pathway
GO:0002833	positive regulation of response to biotic stimulus
GO:0042391	regulation of membrane potential
GO:0022409	positive regulation of cell-cell adhesion
GO:0034329	cell junction assembly
GO:0050767	regulation of neurogenesis
GO:0051962	positive regulation of nervous system development
GO:0006816	calcium ion transport
GO:0048880	sensory system development
GO:0098894	neuron to neuron synapse

ID	Description
GO:0050803	regulation of synapse structure or activity
GO:0050807	regulation of synapse organization
GO:0099173	postsynapse organization
GO:0070588	calcium ion transmembrane transport
GO:0019722	calcium−mediated signaling
GO:0006874	intracellular calcium ion homeostasis
GO:0055074	calcium ion homeostasis
GO:0090596	sensory organ morphogenesis
GO:0050954	sensory perception of mechanical stimulus
GO:0007605	sensory perception of sound

Conclusion

This study reports the first successful construction of a homozygous p.V37I mouse model using CRISPR/Cas9, providing a basis for investigating cochlear function and hearing loss.Mutant mice showed progressive, high-frequency hearing loss, confirmed by elevated auditory brainstem response (ABR) thresholds.No significant cochlear morphology or Connexin26 protein expression changes were observed, as supported by scanning electron microscopy and immunofluorescence results.

Reference

Chen, Y., Hu, L., Wang, X., Sun, C., Lin, X., Li, L., et al. (2016). Characterization of a knock-in mouse model of the homozygous p.V37I variant in Gjb2. Sci. Rep. 6. doi: 10.1038/srep33279

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