A New Mouse Model for Usher Syndrome Crossing Kunming Mice with CBA/J Mice

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Highlights

- A Novel mouse model of USH developed using KM and CBA/J mice.
- KM$^{ush/ush}$ mice exhibit an USH phenotype based on various assessment tools.
- A new mouse model contains Adgrv1 mutations and can be used for audiological research.

Abstract

Background

Previously, we discovered a strain of Kunming mice, referred to as the KM$^{ush/ush}$ strain, that exhibited notably abnormal electroretinogram (ERG) readings and elevated thresholds for auditory brainstem responses (ABRs), which resembled the characteristics of Usher Syndrome (USH). We successfully identified the pathogenic genes, *Pde6b* and *Adgrv1*, after KM$^{ush/ush}$ crossbred with CBA/CaJ mice, referred to as CBA-1$^{ush/ush}$, CBA-2$^{ush/ush}$ or CBA-2$^{ush/ush}$. In this investigation, we crossbred KM$^{ush/ush}$ and CBA/J mice to establish novel recombinant inbred lines and analysed their phenotypic and genotypic characteristics.

Methods

ERG readings, ABR testing, fundus morphology, histological examination of the retina and inner ear, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis, western blotting, DNA sequence analysis and behavioural experiments were performed to assess the phenotypes and genotypes of the progeny lines.

Results

No obvious waveforms in the ERG were detected in F1 hybrid mice while normal ABR results were recorded. The F2 hybrids, which were called $J1^{ush/ush}$ or $J2^{ush/ush}$, exhibited segregated hearing-loss phenotypes. $J1^{ush/ush}$ mice had a retinitis pigmentosa (RP) phenotype with elevated ABR thresholds, whereas $J2^{ush/ush}$ mice exhibited only the RP phenotype. Interestingly, $J1^{ush/ush}$ mice showed significantly higher ABR thresholds than wild-type mice at 28 days post born (P28), and RT-qPCR and DNA-sequencing analysis showed that *Adgrv1* gene expression was significantly altered in $J1^{ush/ush}$ mice, but histological analysis showed no significant structural changes in the organ of Corti or spiral ganglia. Further elevation of ABR-related hearing thresholds by P56 manifested only as a reduced density of spiral ganglion cells, which differed significantly from the previous pattern of cochlear alterations in CBA-$2^{ush/ush}$ mice.

Conclusions

We successfully introduced the hearing-loss phenotype of inbred mice with USH into CBA/J mice, which provides a good animal model for future studies on the important physiological roles of the *Adgrv1* gene in inner-ear structure and for therapeutic studies targeting *Adgrv1*-mutated USH.

Introduction

Usher Syndrome (USH) is a complex disease which comprises a group of autosomal-recessive disorders characterised by retinitis pigmentosa (RP), and various degrees of hearing loss, with or without vestibular abnormalities. As the most common cause of deaf-blindness worldwide, USH has a total prevalence of > 400,000 cases and seriously reduces patients' quality of life (Ehn, 2019). It is essential to develop appropriate medical treatments for USH. However, comprehensive pathogenesis and effective treatments of USH remain unclear until nowadays (Stemerdink, 2022). USH patients are generally classified into three subtypes (USH1, USH2, and USH3) according to the extent of hearing loss, vestibular function, visual-field impairment, disease progression, and age of onset (Géléoc and El-Amraoui, 2020). At present, USH treatment is mainly symptom-based, using hearing aids or cochlear implants to ameliorate sensorineural hearing loss (Hartel, 2017) and visual aids and retinal prostheses to postpone vision loss caused by RP (Fahim, 2018). However, it is reported that the existing therapeutic effects are limited, and none of these treatments can resolve the pathophysiologic mechanisms underlying USH specifically (Toualbi et al., 2020). In the meantime, the non-specificity and variability of clinical manifestations made the clinical diagnosis of USH more complicated. The limitations of this classification are gradually becoming apparent.

Many scientists have confirmed that USH is genetically heterogeneous regarding RP (Nisenbaum, 2022). With the utilisation of next-generation sequencing technology, the list of known pathogenic mutations in USH-related genes has been discovered and expanded. Twelve identified genes are announced corresponding to the clinical subtype (https://hereditaryhearingloss.org) which leads to various gene-specific therapies being developed to treat and cure the symptoms of USH in inner ear and retina (Géléoc and El-Amraoui, 2020), including utilising clustered regularly interspersed short palindromic repeats (CRISPR) tools (Major et al., 2023) and intravitreal injection of an antisense oligonucleotide as a mutation-specific treatment for USH2A. However, the outcomes of these

studies have not yet been formally applied and reported. And more importantly, no widely applicable, safe, and effective treatment method has been developed for treating patients with USH so far. It still takes a long time to develop and test the proper drugs or gene therapies for USH.

Various animal and cellular models have been employed to discover pathogenic mechanisms underlying USH and evaluate the effectiveness of novel therapeutics (Stemerdink, 2022). USH2 is the most common subtype of USH, accounting for over half of all USH patients (Toualbi et al., 2020, Fuster-García, 2021). Three causative genes have been associated with USH2, namely, *Ush2a* (USH2A), *Adgrv1* (USH2C), and *Whrn* (USH2D). In our laboratory, we conducted pre-screening for a naturally occurring mouse model exhibiting both RP and deafness with the Kunming (KM) genetic background. Through this screening, we identified a single strain of mice with USH phenotypes, designated as $KM^{ush/ush}$ mice (Yao, 2016). To investigate the genetic background of $KM^{ush/ush}$ mice and the causes of their auditory and ocular impairments, we crossed $KM^{ush/ush}$ mice with CBA/CaJ mice, which were control mice with normal hearing (Zheng et al., 1999), to distinguish the ocular and auditory phenotypes and genotypes. The results showed that retinal degeneration in $KM^{ush/ush}$ mice was caused by a *Pde6b* mutation, a well-known gene in the development and progression of RP (Yao, 2016), whereas auditory impairment was caused by an *Adgrv1* mutation. The resulting CBA-$2^{ush/ush}$ mice represent an animal model for inheritable hearing loss due to a mutation in the Usher Syndrome 2C gene, *Adgrv1*. That was the first report of a mouse strain with hearing loss being isolated from an RP phenotype, and this animal model can be used to study the pathological mechanisms of RP/USH. *Adgrv1* mutations are present in approximately 5-19 % of clinical cases, second only to *Ush2a* (Bonnet, 2016). *Adgrv1* is expressed in several tissues, including the brain, lungs, kidneys, eyes, and inner ear (Weston, 2004), and expression of the encoded protein product, *Vlgr1*, is sensitive to changes in the extracellular Ca^{2+} concentration in hair and photoreceptor cells. Defects in *Vlgr1* may cause imbalances in the extramembrane Ca^{2+} concentrations of both of these cell types (Krzysko, 2022), although the exact mechanism remains unclear. To date, the literature documents only five mouse models of deafness definitively attributed to *Adgrv1* mutations (Stemerdink, 2022), thereby significantly constraining research into its underlying

mechanisms. As a result, this mouse model can also be applied to discover the pathogenesis and innovative therapy of *Adgrv1* gene (Yan, 2018).

In our previous research, the cochleae of CBA-2$^{ush/ush}$ mice presented obvious morphological changes at early stage, which is unsuitable for developing drugs or therapies for USH. Furthermore, subsequent breeding between CBA-2$^{ush/ush}$ and CBA-3$^{ush/ush}$ mice showed inbreeding-related declines, which greatly hindered the subsequent study of the pathological mechanisms of RP/USH. A new mouse model is required for further research regarding USH. CBA/J is regarded as another “normal-hearing” controls for hearing and deafness research (McGinn et al., 1992), although it is different from CBA/CaJ in the pattern of inner ear damage of sensorineural deafness (Ohlemiller et al., 2010) and the visual function. Therefore, in this study, we crossed KM$^{ush/ush}$ mice with CBA/J mice, introducing the pathogenic gene from KM$^{ush/ush}$ mice into the CBA/J. Firstly, we used functional, morphological, and other technical methods to confirm the presence of USH phenotype in KM$^{ush/ush}$. Then we crossed CBA/J mice with KM$^{ush/ush}$ mice and inbred the F1 hybrid mice. Two phenotypic segregations were observed in F2 hybrids, including one characterised by electroretinograms (ERGs) without waveforms and another by elevated auditory brainstem response (ABR) thresholds. New mice with the CBA/J background were bred and both the phenotype and genotype in the offspring were identified. These mice represent a novel animal model for studying *Adgrv1* mutations within the CBA/J background for further research in USH syndrome and for advancing audiology research in general.